US008263358B2

(12) **United States Patent**
Clark et al.

(10) **Patent No.: US 8,263,358 B2**
(45) **Date of Patent: Sep. 11, 2012**

(54) **INTRACELLULAR NANOSENSORS AND METHODS FOR THEIR INTRODUCTION INTO CELLS**

(75) Inventors: **Heather A. Clark**, Lexington, MA (US); **Daniel I. Harjes**, Allston, MA (US); **John M. Dubach**, Somerville, MA (US)

(73) Assignee: **The Charles Stark Draper Laboratory, Inc.**, Cambridge, MA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 658 days.

(21) Appl. No.: **12/287,937**

(22) Filed: **Oct. 14, 2008**

(65) **Prior Publication Data**

US 2009/0142274 A1 Jun. 4, 2009

Related U.S. Application Data

(60) Provisional application No. 60/999,062, filed on Oct. 15, 2007.

(51) **Int. Cl.**

C12Q 1/02	(2006.01)
C12M 1/00	(2006.01)
G01N 33/53	(2006.01)
G01N 33/52	(2006.01)
A61K 49/00	(2006.01)
B82B 3/00	(2006.01)

(52) **U.S. Cl.** **435/29**; 424/9.6; 435/7.1; 435/288.7; 977/774; 977/927

(58) **Field of Classification Search** 424/9.6; 435/7.2, 29, 288.7; 977/774, 927
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

6,143,570	A	11/2000	Alder et al.
7,704,407	B2	4/2010	Makino et al.
2004/0048390	A1	3/2004	Wang et al.
2006/0083688	A1	4/2006	Singaram et al.
2006/0148104	A1	7/2006	Marini et al.
2007/0107625	A1	5/2007	Anderson et al.
2008/0131909	A1	6/2008	Clark et al.
2009/0155183	A1	6/2009	Clark
2010/0221188	A1	9/2010	Clark et al.
2010/0227334	A1	9/2010	Clark

FOREIGN PATENT DOCUMENTS

WO	WO-2004/083902		9/2004
WO	WO-2006/050257		5/2006
WO	WO 2007/054689	A1	5/2007
WO	WO-2007/067733		6/2007
WO	WO 2007/067743	A2	6/2007
WO	WO-2008/016646		2/2008
WO	WO-2008/063151		5/2008

OTHER PUBLICATIONS

Bruchez et al., "Semiconductor Nanocrystals as Fluorescent Biological Labels," *Science* 281:2013-2016 (1998).

Chen et al., Lighting up cancer cells with "dots", *The Lancet*, 364:2001-2003 (2004).

Clark, H. A., et al., "Optochemical Nanosensors and Subcellar Applications in Living Cells," *Mikrochimica Acta*, 131(1/02):121-128 (1999).

Jia et al., "A Method to Construct a Third-Generation Horseradish Peroxidase Biosensor: Self-Assembling Gold Nanoparticles to Three-Dimensional Sol—Gel Network", *Anal. Chem* 74:2217-2223 (2002).

Puntener et al., "Imporving the lower detection limit of potentiometric sensors by covalently binding the ionophore to a polymer backbone," *Analytica Chimica Acta*, 503:187-194, (2004).

Springsteen et al., "Alizarin Red S. as a general optical reporter for studying the binding of boronic acids with carbohydrates", *The Royal Society of Chemistry* 17:1608-1609 (2001).

Buck et al., "Nanoscale Probes Encapsulated by Biologically Localized Embedding (PEBBLEs) for Ion Sensing and Imaging in Live Cells", *TALANTA*, 63:1 pp. 41-59 (2004).

Clapp, et al., "Quantum Dot-Based Multiplexed Fluorescence Resonance Energy Transfer," *J. Am. Chem. Soc.*, 127:18212-18221 (2005).

Dubach, et al., "Ion-Selective Nano-optodes Incorporating Quantum Dots," *J. Am. Chem. Soc.*, 129(27), 8418-8419, (2007).

Goldman, et al., "Multiplexed Toxin Analysis Using Four Colors of Quantum Dot Fluororeagents," *Analytical Chemistry*, 76(3):684-688 (2004).

Nagai et al., "Circularly permuted green flourescent proteins engineered to sense Ca^{2+}", *PNAS*, 98(6):3197-3202 (2001).

Ruedas-Rama, et al., "A multi-ion particle sensor," *Chem. Commun. (Camb)*, 15:1544-1546 (2007).

Snee, et al., "A Ratiometric CdSe/ZnS Nanocrystal pH Sensor," *J. Am. Chem. Soc.*, 128(41):13320-13321 (2006).

Xu, et al., "Multicolor Quantum Dot Encoding for Polymeric Particle-Based Optical Ion Sensors," *Analytical Chemistry*, 79(10):3716-3723 (2007).

International Search Report, PCT/US2008/011726, dated Mar. 16, 2009.

Garg et al., "Micropigmentation: Tattooing for Medical Purposes", *Dermatol. Surg.* 31:928-31 (2005).

(Continued)

Primary Examiner — Jon P Weber
Assistant Examiner — Kailash C Srivastava
(74) *Attorney, Agent, or Firm* — Foley & Lardner LLP; Stephanie H. Vavra

(57) **ABSTRACT**

The invention provides ion-selective sensors capable of selectively measuring ions, e.g., Na^+, K^+, Cl^-, etc., in the cytosol of a single living cell. The sensor comprises one or more quantum dots or a fluorescent dye, a pH-sensitive dye, and optionally an ion-selective component such as an ionophore. These elements may, for example, be disposed in a polymer matrix. The polymer matrix comprises an internalizing moiety which enables the sensor to localize within the cytosol of a cell. The internalizing moiety comprises a small molecule or peptide such as an amine, antepennepedia, mastoparan, or melittin that react under acidic conditions to release a sensor from the confines of a endosome. Once in the cytosol the sensors may detect ionic analytes by selective ion extraction by the polymer, thereby inducing a pH change within the sensor which in turn changes the absorbance of the pH-sensitive dye. The change of absorbance may in turn attenuate the intensity of detectable emissions, e.g., fluorescence, from the quantum dot or dye by directly absorbing its fluorescence emission.

62 Claims, 11 Drawing Sheets

OTHER PUBLICATIONS

Kulcu et al., "Physiological Differences Between Interstitial Glucose and Blood Glucose Measured in Human Subjects", *Diabetes Care*, 26(8):2405-09 (2003).

Schmidtke et al., "Measurement and Modeling of the Transient Difference Between Blood and Subcutaneous Glucose Concentrations in the Rat After Injection of Insulin", *Proc. Natl. Acad. Sci. USA*, 95:294-99 (1998).

Tamada et al., "Noninvasive Glucose Monitoring Comprehensive Clinical Results", *JAMA*, 282(19):1839-44 (1999).

Arimori et al., "A D-glucose Selective Fluorescent Assay," *Tetrahedron Letters*, 43:303-305 (2002).

Russell et al., "A Fluorescence-Based Glucose Biosensor Using Concanavalin A and Dextran Encapsulated in a Poly(ethylene glyco) Hydrogel," *Anal. Chem*. 71:3126-3132 (1999).

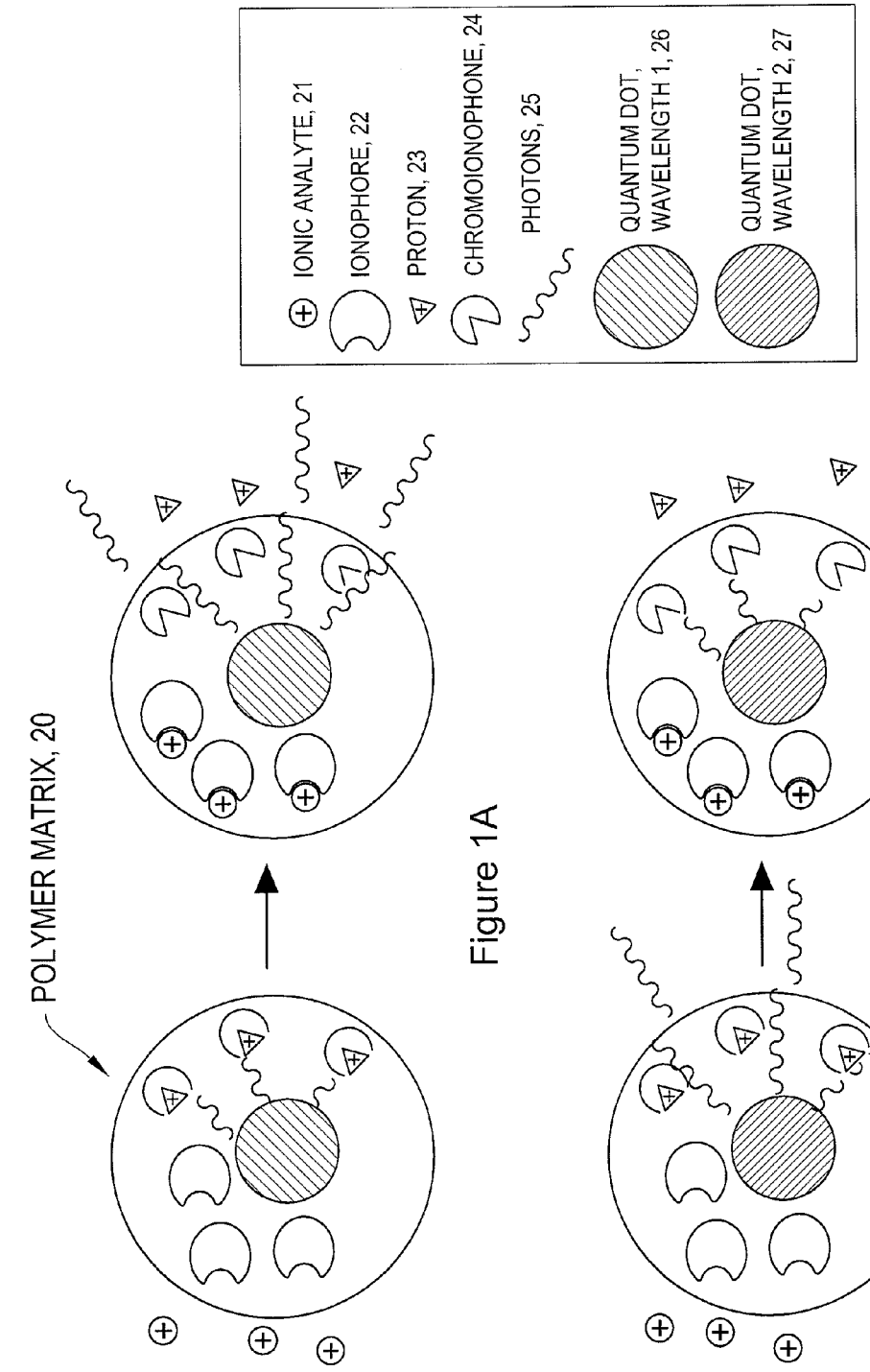

Figure 1A

Figure 1B

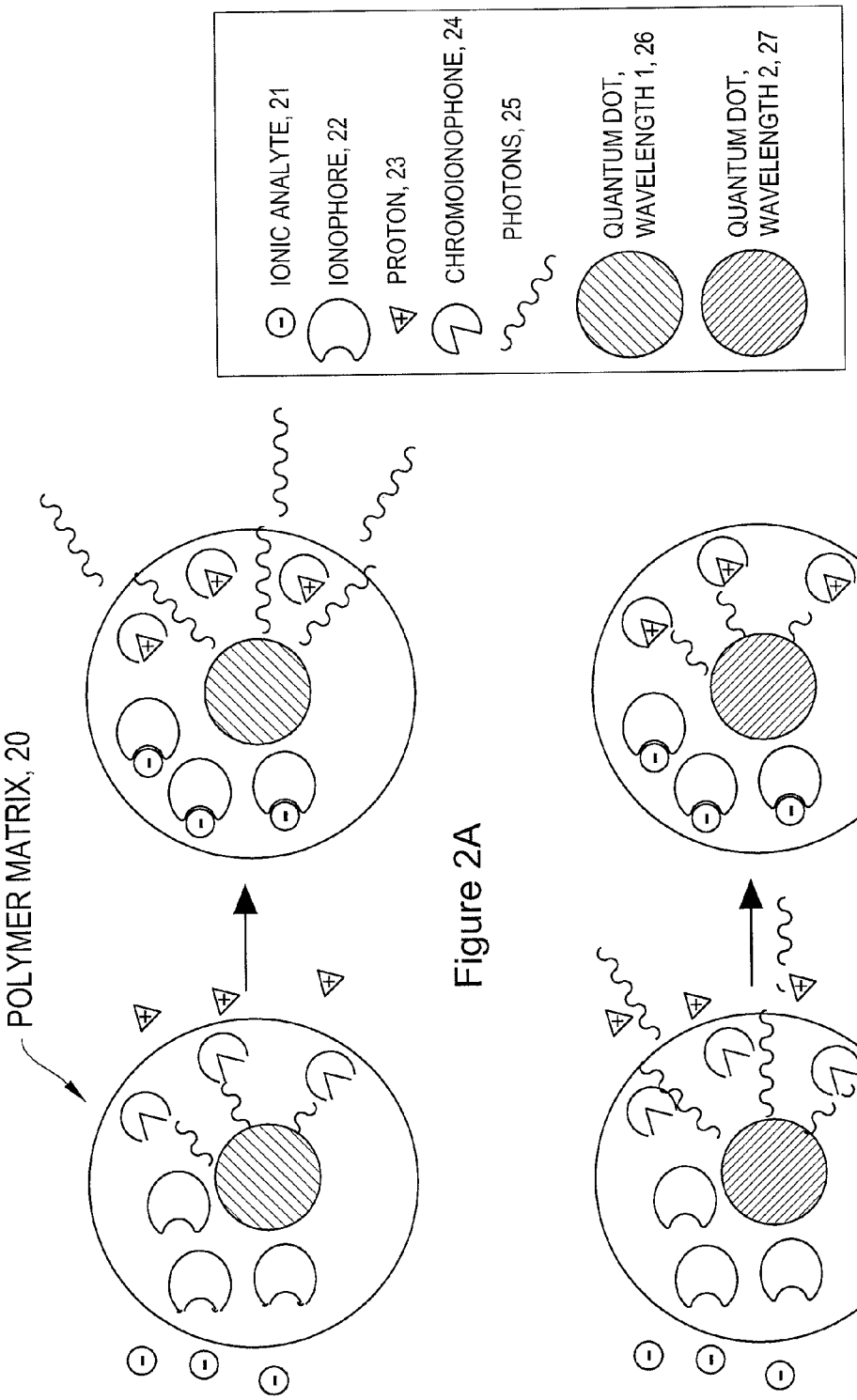

Figure 2A

Figure 2B

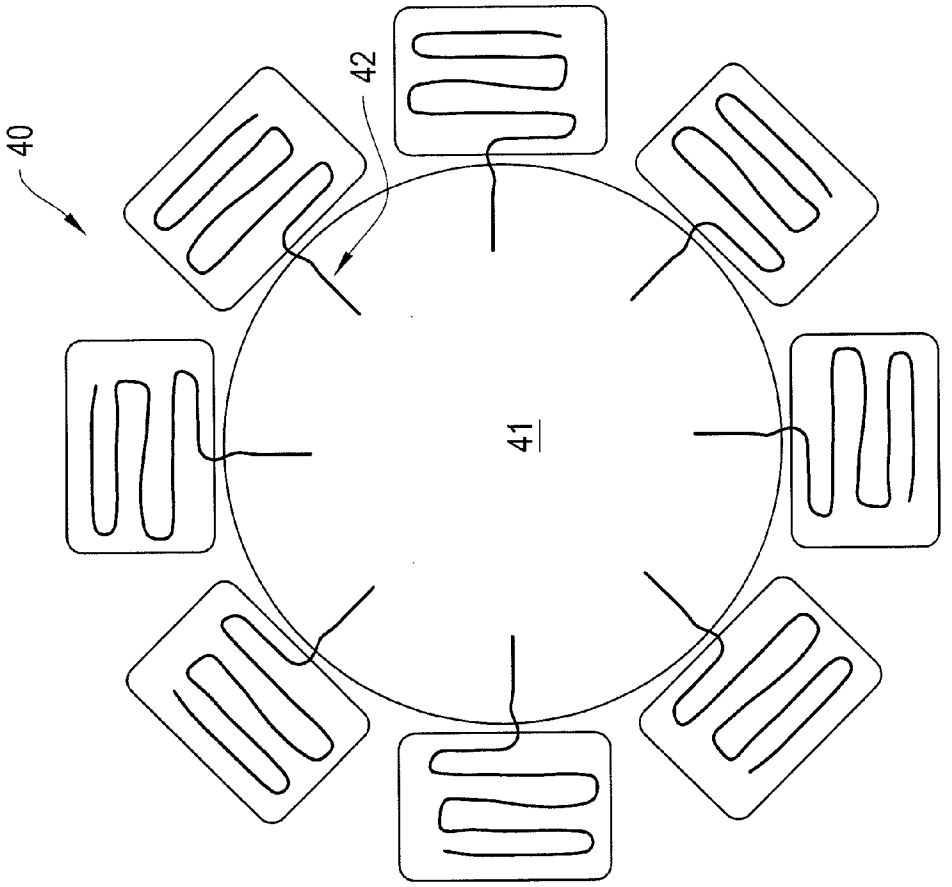

Figure 3

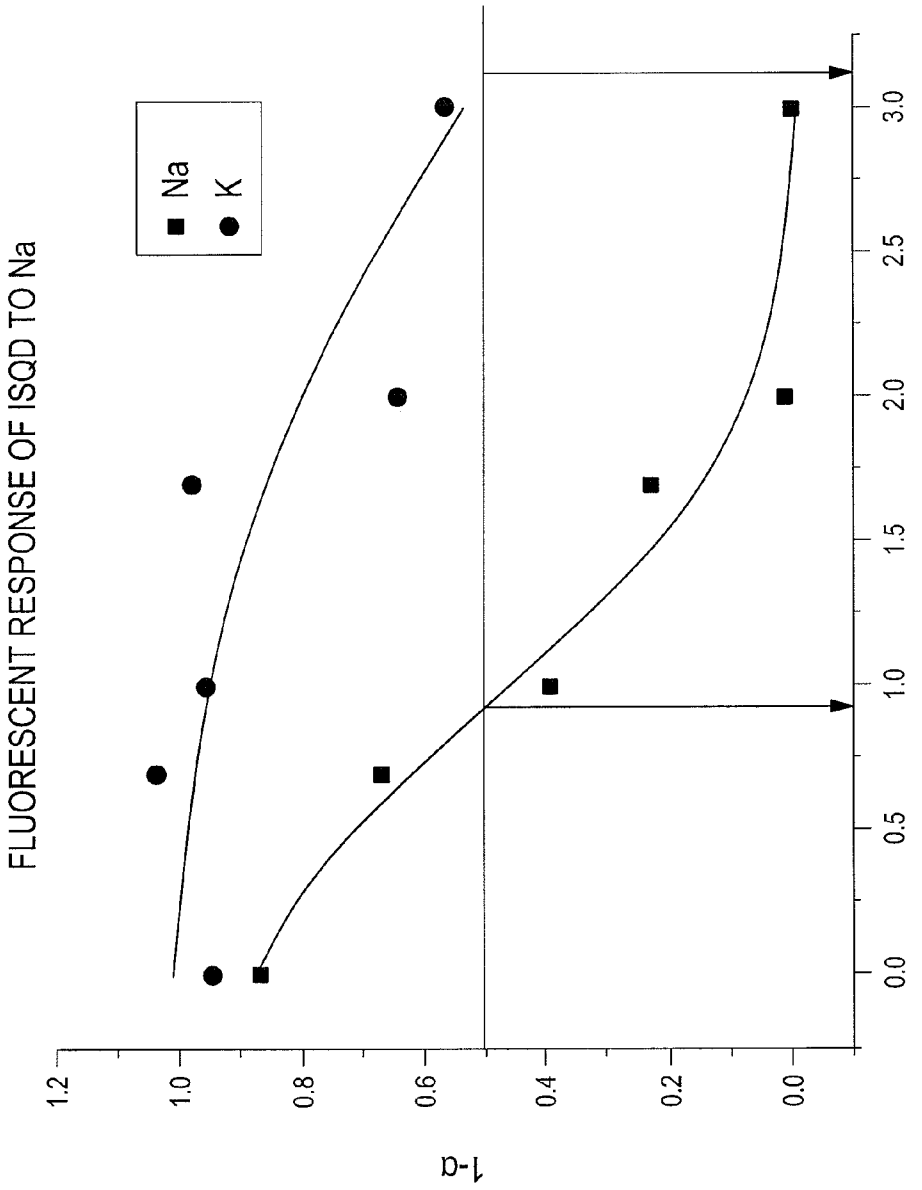
FLUORESCENT RESPONSE OF ISQD TO Na
Na
K
1-α
log[ION] mM
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0.0
0.5
1.0
1.5
2.0
2.5
3.0

Figure 4

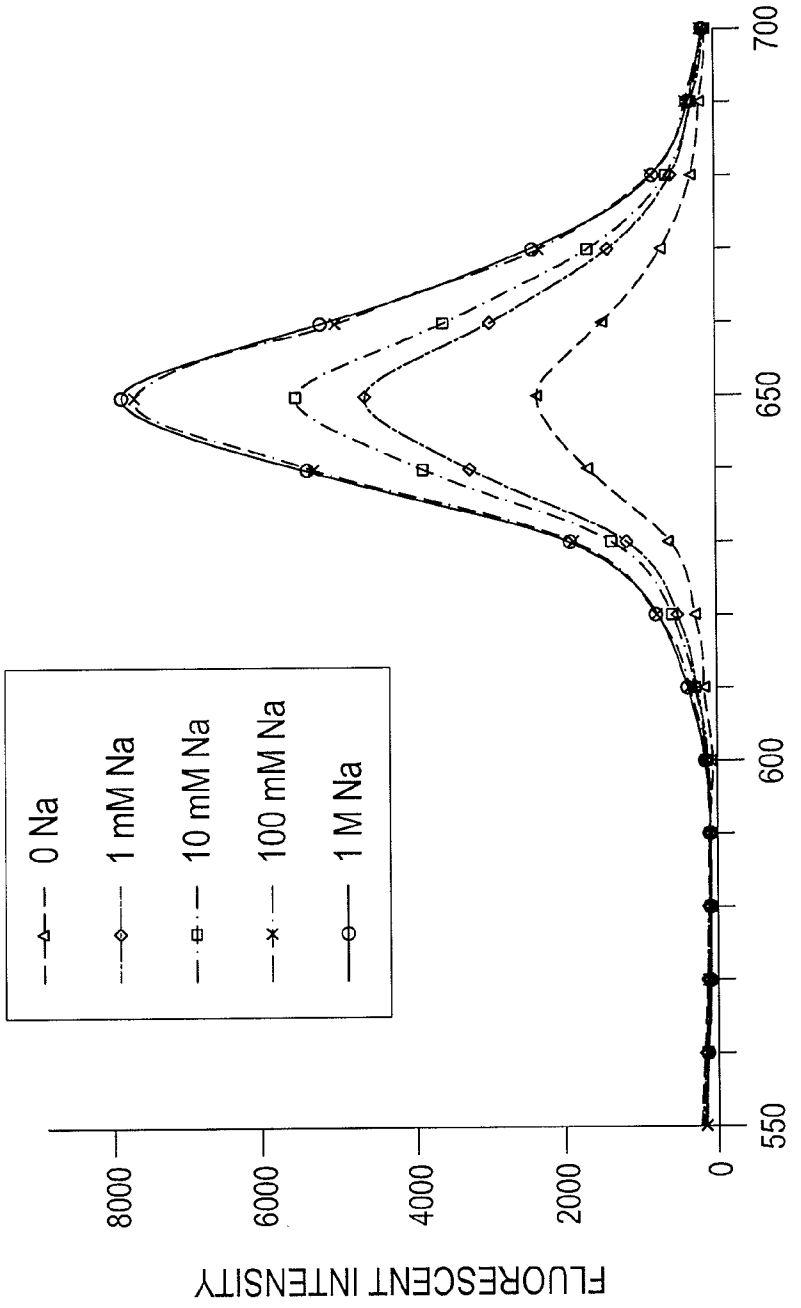
FLUORESCENT INTENSITY
8000
6000
4000
2000
0
550
600
650
700
EMISSION WAVELENGTH
0 Na
1 mM Na
10 mM Na
100 mM Na
1 M Na

Figure 5A

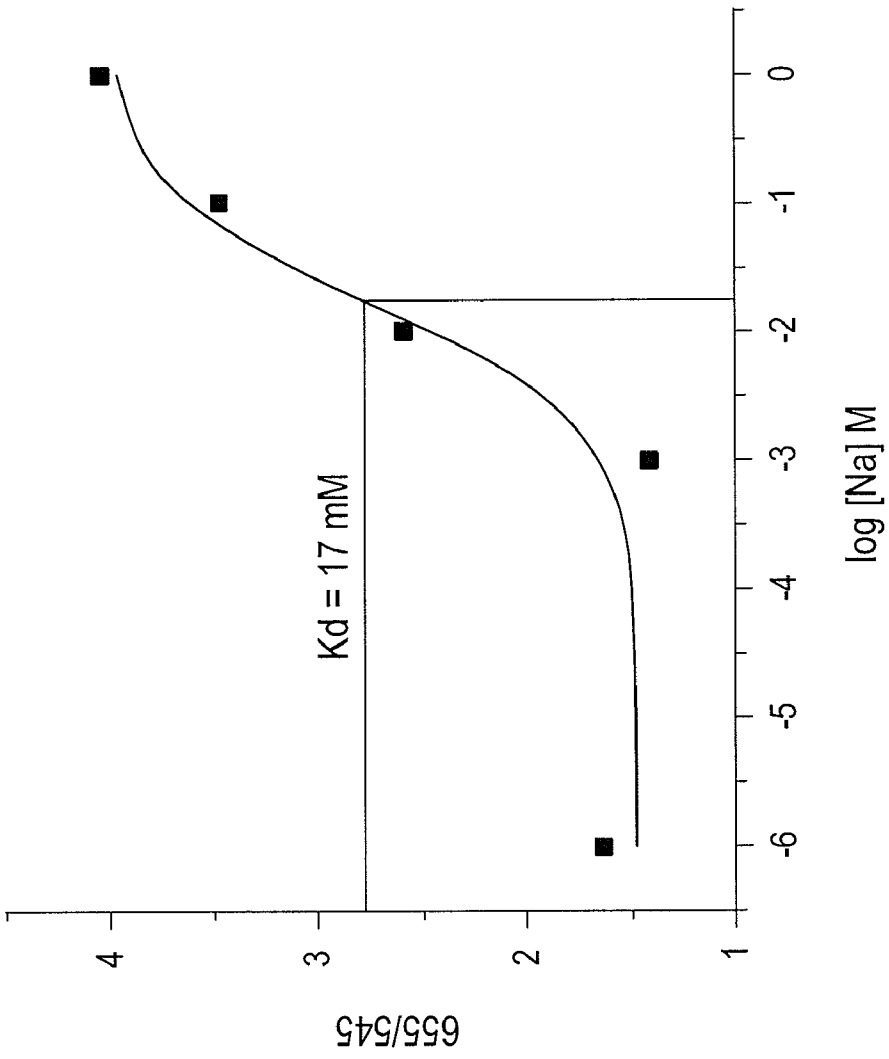

Figure 5B

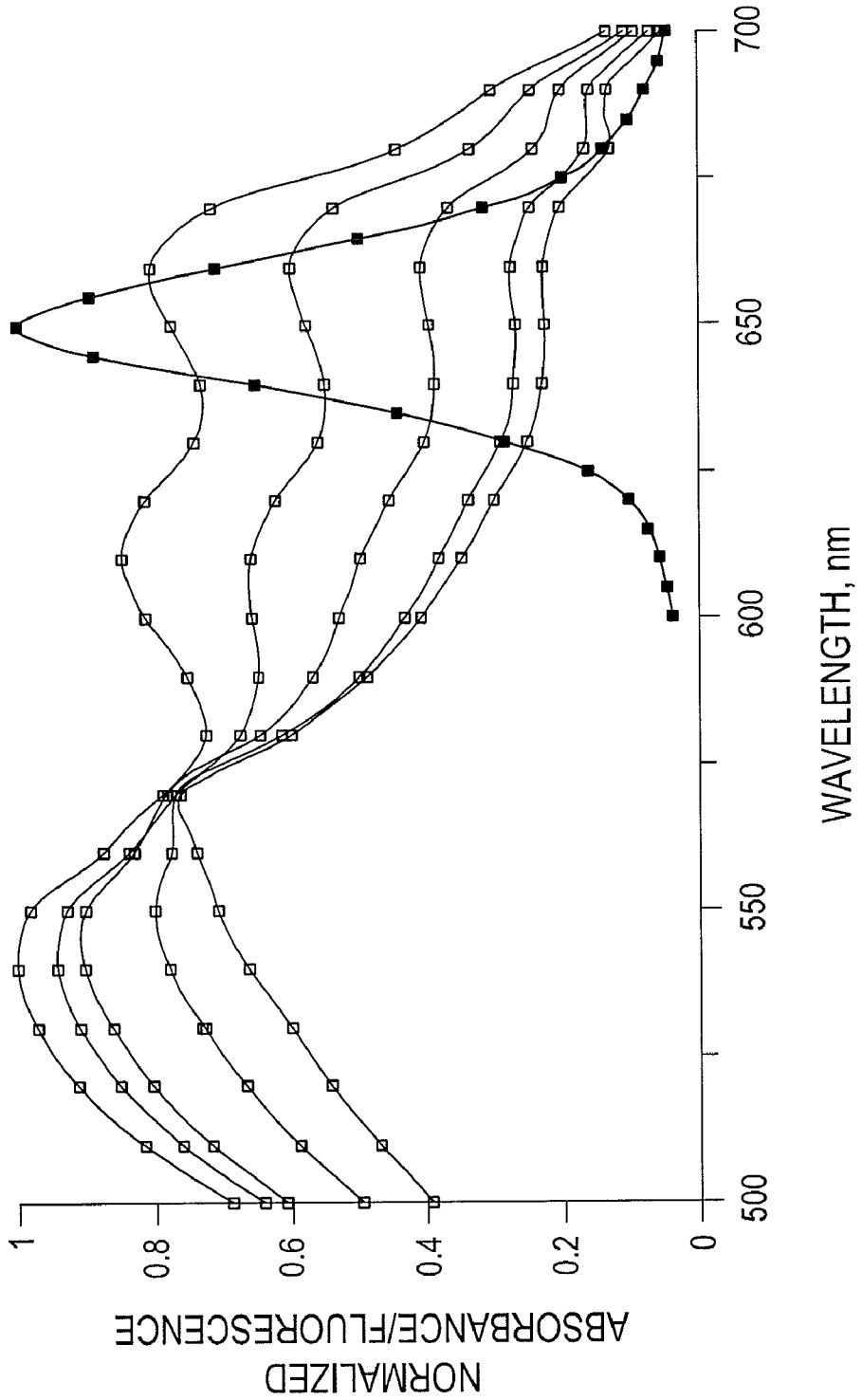
NORMALIZED
ABSORBANCE/FLUORESCENCE
0
0.2
0.4
0.6
0.8
1
500
550
600
650
700
WAVELENGTH, nm

Figure 6

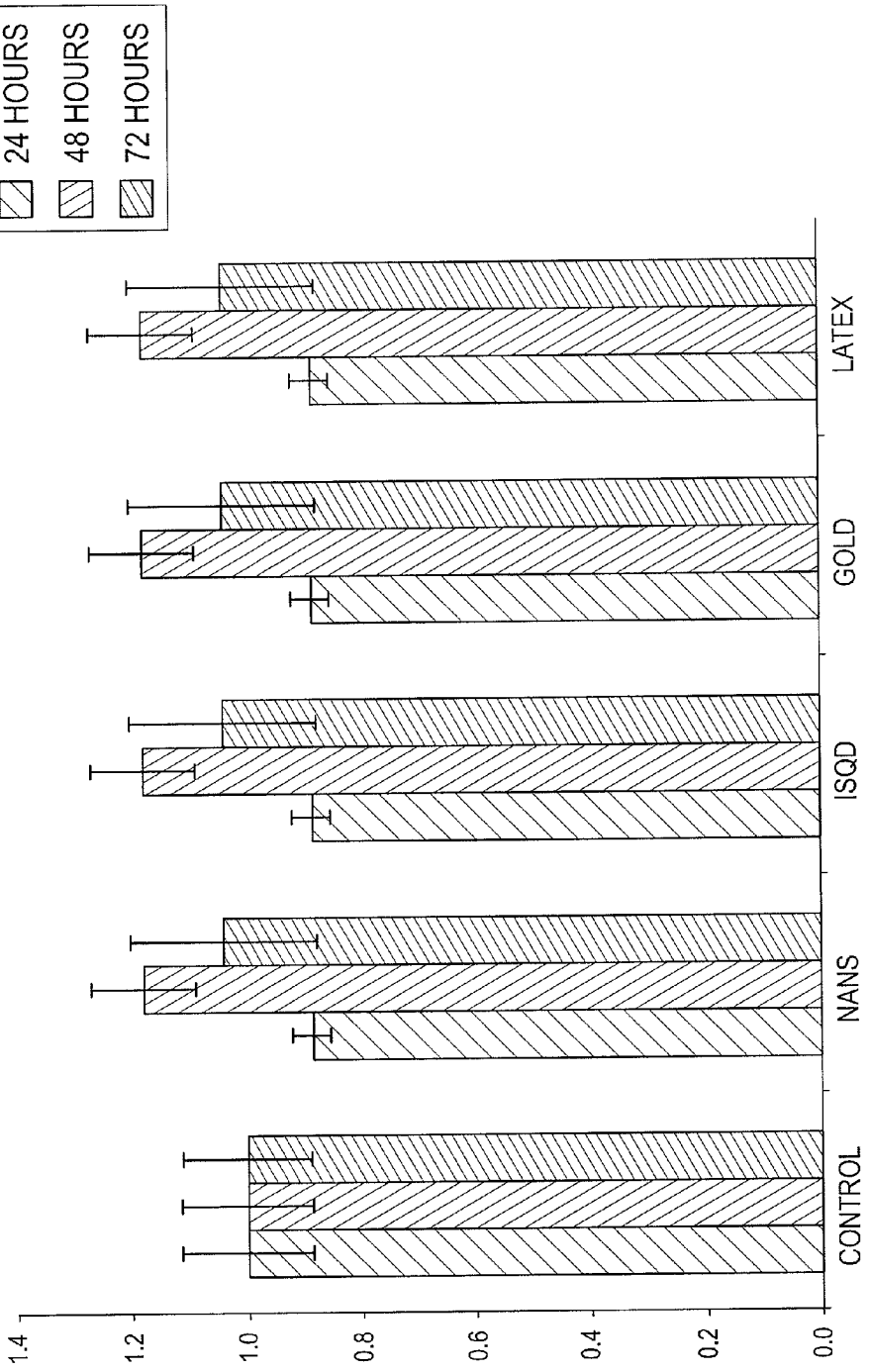
24 HOURS
48 HOURS
72 HOURS
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
CONTROL
NANS
ISQD
GOLD
LATEX

Figure 7

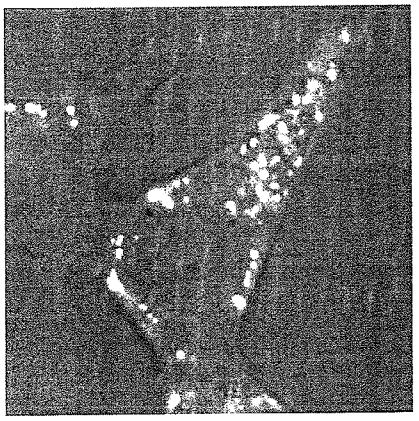

Figure 8

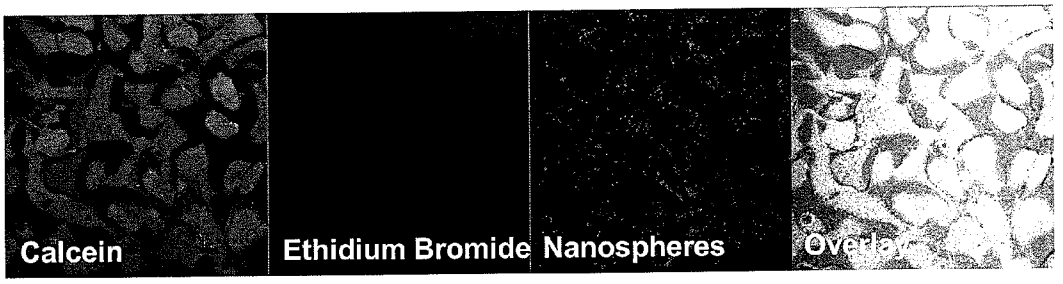

Figure 9

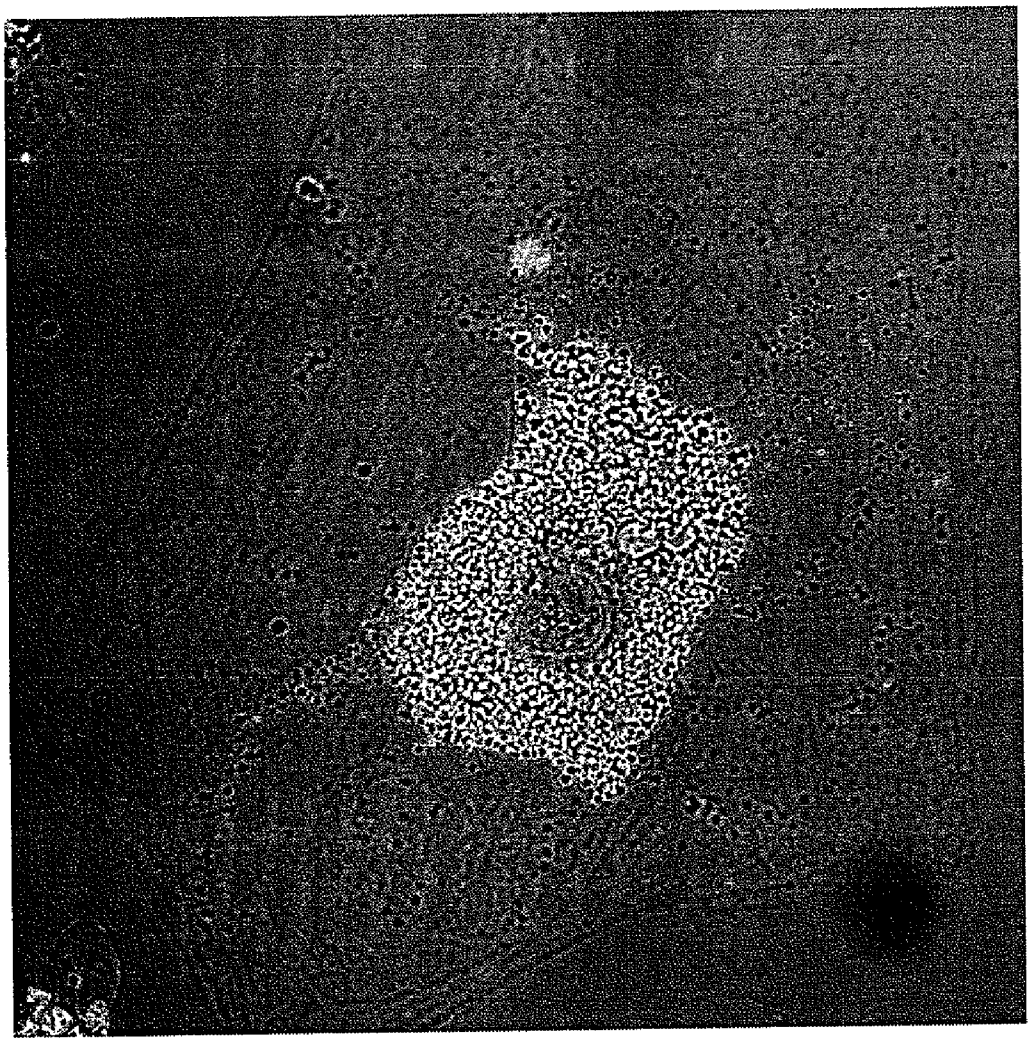

Figure 10A

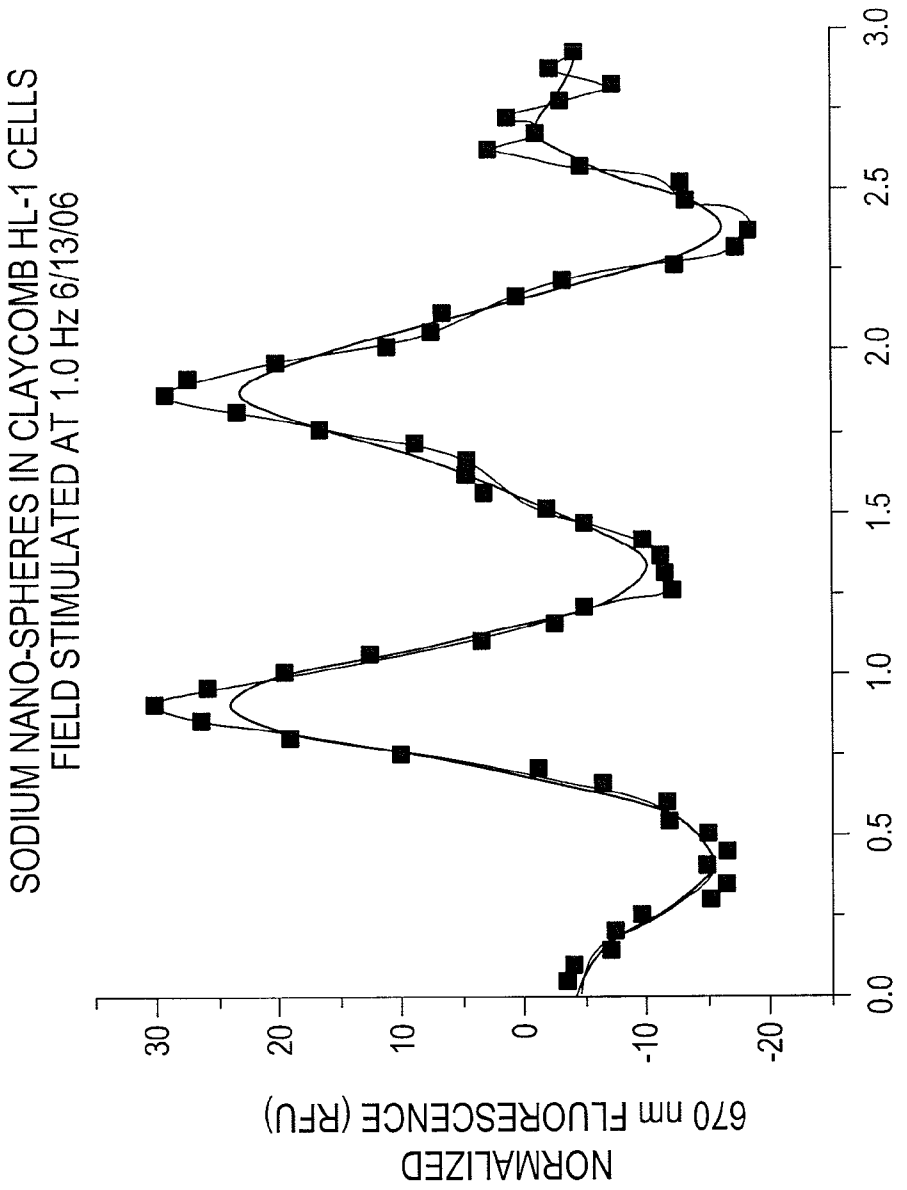
SODIUM NANO-SPHERES IN CLAYCOMB HL-1 CELLS
FIELD STIMULATED AT 1.0 Hz 6/13/06
NORMALIZED
670 nm FLUORESCENCE (RFU)
30
20
10
0
-10
-20
0.0
0.5
1.0
1.5
2.0
2.5
3.0
TIME (SEC)

Figure 10B

INTRACELLULAR NANOSENSORS AND METHODS FOR THEIR INTRODUCTION INTO CELLS

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 60/999,062 filed Oct. 15, 2007, which application is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

Intra-cellular sensors offer a powerful tool for understanding the mechanisms within a cell. Such sensors can detect the presence or concentration of an analyte within the cell, and when multiple sensors are distributed within the interior of the cell, the presence of analytes in relation to different cellular organelles and the cell membrane can be better understood.

Sensors can be introduced into cells using a variety of techniques, however, in many cases, the sensor becomes encapsulated within an endosome of a cell. In the endosome, the sensor is sequestered from the analytes in the cytosol, rendering the sensor ineffective for monitoring intracellular analytes or otherwise diminishing its sensitivity. In order to localize sensors in the cytosol of the cell, methods or compositions that allow the sensor to escape from the endosome into the cytosol of the cell would be highly desirable.

SUMMARY OF THE INVENTION

The invention provides ion-selective sensors capable of selectively measuring ions, e.g., Na^+, K^+, Cl^-, etc., in the cytosol of a single living cell. The sensor comprises a signal source (e.g., one or more quantum dots or a fluorescent dye), a pH-sensitive dye, and optionally an ion-selective component such as an ionophore. These elements may, for example, be disposed in a polymer matrix. The polymer matrix comprises an internalizing moiety which assists the sensor in localizing within the cytosol of a cell. In certain embodiments, the internalizing moiety comprises a small molecule or peptide, such as an amine, that reacts, e.g., under acidic conditions, to release a sensor from the confines of a endosome. Once in the cytosol, the sensors may detect ionic analytes by selective ion extraction by the polymer, thereby inducing a pH change within the sensor which in turn changes the absorbance of the pH-sensitive dye. The change of absorbance may in turn attenuate the intensity of detectable emissions, e.g., fluorescence, from the quantum dot or dye by directly absorbing its fluorescence emission.

BRIEF DESCRIPTION OF DRAWINGS

FIG. **1** is a representation of two exemplary modes of operation of the quantum dot incorporated sensor for the detection of cationic analytes. In Mode A, the sensor fluoresces in the presence of the ionic analyte. In Mode B, the sensor fluoresces in the absence of ionic analyte.

FIG. **2** is a representation of two exemplary modes of operation of the quantum dot incorporated sensor for the detection of anionic analytes. In Mode A, the sensor fluoresces in the presence of the ionic analyte. In Mode B, the sensor fluoresces in the absence of ionic analyte.

FIG. **3** is a representation of a sensor coated with a surface modifier such as PEG.

FIG. **4** shows the selectivity of the nanosensor of the invention for ion detection.

FIG. **5** depicts the experimental response to sodium. A) Spectral response of immobilized sensors to increasing concentrations of sodium. B) Calibration curve of ratiometric sensors.

FIG. **6** depicts the spectral overlap of a quantum dot that fluoresces at 655 nm and the absorbance of a chromoionophore at varying sodium concentrations.

FIG. **7** depicts biocompatibility of nanosensors in HEK cells. HEK cells were incubated with either control (water), nanosensors without quantum dots (nans), quantum dot nanosensors, 100 nm gold nanoparticles, or 20 nm latex beads (a negative control).

FIG. **8** is a confocal image of nanosensors without quantum dots loaded into an HEK 293 cell.

FIG. **9** shows a LIVE/DEAD assay wherein nanosensors with quantum dots were loaded into HEK 293 cells overnight and then stained. The green indicates healthy cells, while the red stains the nuclei of dead cells. No difference in the ratio of live to dead cells was noted between nanosensor loaded cells and control (no nanosensors).

FIG. **10** depicts A. fluorescence image of an isolated neonatal rat ventricular myocyte loaded with sodium-selective nanosensors. B. the fluorescence collected from a nanosensor in a cardiac cell during stimulation

DETAILED DESCRIPTION OF THE INVENTION

In brief overview, embodiments of the present invention provide systems, methods, and devices for measuring ionic analytes. In exemplary embodiments, sensors are placed inside or outside a cell. Emissions from the sensor indicate the ion concentrations and fluxes from the cell. In certain aspects, the sensors comprise a polymer, a fluorescent semiconductor nanocrystal (also known as a Quantum Dot™ particle) or a fluorescent dye that fluoresces at a first wavelength, and a chromoionophore that absorbs photons of the first wavelength in one state and does not absorb photons of the first wavelength in a second state. In monitoring ionic analytes, the chromoionophore changes state in response to proton concentration (i.e., the protonated chromoionophore is one state while the deprotonated chromoionophore is a second state). To monitor a specific analyte, an ionophore that selectively associates with specific ions or groups of ions is included in the sensor. Once the ionophore associates with a cationic analyte (e.g., Na^+ associates with a Na^+-selective ionophore), for example, protons are displaced from the sensor to equilibrate charge, altering the state of the chromoionophore. The fluorescence emitted from the sensor indicates the state of the chromoionophore which correlates to the presence and/or concentration of the ionic analyte. Sensors that use fluorescent dyes instead of quantum dots are disclosed in U.S. patent application Ser. No. 11/522,169, filed Sep. 15, 2006, the disclosure of which is incorporated herein by reference.

In certain embodiments, the sensor includes an ionophore, a chromoionophore, a quantum dot, and optionally one or more additives. The components are typically embedded in a polymer. In certain embodiments, the polymer comprises poly(caprolactone) (PCL), ethylene vinyl acetate polymer (EVA), poly(lactic acid) (PLA), poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), poly(lactic acid-co-glycolic acid) (PLGA), poly(L-lactic acid-co-glycolic acid) (PLLGA), poly (D,L-lactide) (PDLA), poly(L-lactide) (PLLA), poly(D,L-lactide-co-caprolactone), poly(D,L-lactide-co-caprolactone-co-glycolide), poly(D,L-lactide-co-PPO-co-D,L-lactide), poly(D,L-lactide-co-PPO-co-D,L-lactide), polyalkyl cyanoacralate, polyurethane, poly-L-lysine (PLL), hydrox-

ypropyl methacrylate (HPMA), polyethyleneglycol, poly-L-glutamic acid, poly(hydroxy acids), polyanhydrides, polyorthoesters, poly(ester amides), polyamides, poly(ester ethers), polycarbonates, silicones, polyalkylenes such as polyethylene, polypropylene, and polytetrafluoroethylene, polyalkylene glycols such as poly(ethylene glycol) (PEG), polyalkylene oxides (PEO), polyalkylene terephthalates such as poly(ethylene terephthalate), polyvinyl alcohols (PVA), polyvinyl ethers, polyvinyl esters such as poly(vinyl acetate), polyvinyl halides such as poly(vinyl chloride) (PVC), polyvinylpyrrolidone, polysiloxanes, polystyrene (PS), polyurethanes, derivatized celluloses such as alkyl celluloses, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, hydroxypropylcellulose, carboxymethylcellulose, polymers of acrylic acids, such as poly(methyl(meth) acrylate) (PMMA), poly(ethyl(meth)acrylate), poly(butyl (meth)acrylate), poly(isobutyl(meth)acrylate), poly(hexyl (meth)acrylate), poly(isodecyl(meth)acrylate), poly(lauryl (meth)acrylate), poly(phenyl(meth)acrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate) (jointly referred to herein as "polyacrylic acids"), and copolymers and mixtures thereof, polydioxanone and its copolymers, polyhydroxyalkanoates, poly (propylene fumarate), polyoxymethylene, poloxamers, poly (ortho)esters, poly(butyric acid), poly(valeric acid), poly (lactide-co-caprolactone), trimethylene carbonate, polyvinylpyrrolidone, and the polymers described in Shieh et al., 1994, J. Biomed. Mater. Res., 28, 1465-1475, and in U.S. Pat. No. 4,757,128, Hubbell et al., U.S. Pat. Nos. 5,654,381; 5,627,233; 5,628,863; 5,567,440; and 5,567,435. Other suitable polymers include polyorthoesters (e.g. as disclosed in Heller et al., 2000, Eur. J. Pharm. Biopharm., 50:121-128), polyphosphazenes (e.g. as disclosed in Vandorpe et al., 1997, Biomaterials, 18:1147-1152), and polyphosphoesters (e.g. as disclosed in Encyclopedia of Controlled Drug Delivery, pp. 45-60, Ed. E. Mathiowitz, John Wiley & Sons, Inc. New York, 1999), as well as blends and/or block copolymers of two or more such polymers. The carboxyl termini of lactide- and glycolide-containing polymers may optionally be capped, e.g., by esterification, and the hydroxyl termini may optionally be capped, e.g., by etherification or esterification. In certain embodiments, the polymer comprises or consists essentially of polyvinyl chloride (PVC), polymethyl methacrylate (PMMA) and decyl methacrylate or copolymers or any combination thereof.

In certain embodiments, the polymer comprises a biocompatible polymer, e.g., selected from poly(caprolactone) (PCL), ethylene vinyl acetate polymer (EVA), poly(ethylene glycol) (PEG), poly(vinyl acetate) (PVA), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(lactic-co-glycolic acid) (PLGA), polyalkyl cyanoacrylate, polyethylenimine, dioleyltrimethyammoniumpropane/dioleyl-sn-glycerol-phosphoethanolamine, polysebacic anhydrides, polyurethane, nylons, or copolymers thereof. In polymers including lactic acid monomers, the lactic acid may be D-, L-, or any mixture of D- and L-isomers. The terms "biocompatible polymer" and "biocompatibility" when used in relation to polymers are art-recognized. For example, biocompatible polymers include polymers that are neither themselves toxic to the host (e.g., a cell, an animal, or a human), nor degrade (if the polymer degrades) at a rate that produces monomeric or oligomeric subunits or other byproducts at toxic concentrations in the host. Consequently, in certain embodiments, toxicology of a biodegradable polymer intended for intracellular or in vivo use, such as implantation or injection into a patient, may be determined after one or more toxicity analyses. It is not necessary that any subject composition have a purity of 100% to be deemed biocompatible. Hence, a subject composition may comprise 99%, 98%, 97%, 96%, 95%, 90% 85%, 80%, 75% or even less of biocompatible polymers, e.g., including polymers and other materials and excipients described herein, and still be biocompatible.

The polymer phase may comprise a plasticizer, such as dioctyl sebacate (DOS), o-nitrophenyl-octylether, dimethyl phthalate, dioctylphenyl-phosphonate, dibutyl phthalate, hexamethylphosphoramide, dibutyl adipate, dioctyl phthalate, diundecyl phthalate, dioctyl adipate, dioctyl sebacate, or other suitable plasticizers. In certain embodiments, the plasticizer is poly(glycerol sebacate), PGS.

In certain embodiments, e.g., particularly where the polymer is biocompatible, a biocompatible plasticizer is used. The term "biocompatible plasticizer" is art-recognized, and includes materials which are soluble or dispersible in the relevant polymer, which increase the flexibility of the polymer matrix, and which, in the amounts employed, are biocompatible. Suitable plasticizers are well known in the art and include those disclosed in U.S. Pat. Nos. 2,784,127 and 4,444,933. Specific plasticizers include, by way of example, acetyl tri-n-butyl citrate (c. 20 weight percent or less), acetyltrihexyl citrate (c. 20 weight percent or less), butyl benzyl phthalate, dibutylphthalate, dioctylphthalate, n-butyryl tri-n-hexyl citrate, diethylene glycol dibenzoate (c. 20 weight percent or less) and the like.

The ionophore is a compound, typically an electrically neutral compound, that associates (e.g., forms a complex, chelate, or other non-covalent association) with a target ion, and is selective for the target ion relative to other ions. The ionophore is selected to be lipid-soluble and does not emit light in the visible spectrum in either of its complexed and non-complexed states. In certain aspects, the ionophore of the mixture included herein is chosen to selectively bind an ionic analyte, for example, K^+, Na^+, Ca^{2+}, H^+, Ba^{2+}, Li^+, Cl^-, NH_4^+, or NO_3^-. Potassium ion ionophores include, for example, valinomycin, crown ethers, e.g., dimethyldibenzo-30-crown-10, dicyclohexyl-18-crown, dimethyldicyclohexyl-18-crown-6, tetraphenyl borate, tetrakis(chlorophenyl) borate. Sodium ion ionophores include, for example, methyl monensin, N,N',N"-triheptyl-N,N',N"-trimethyl-4,4',4"-propylidintris-(3-oxabutyramide), N,N,N',N'-tetracyclohexyl-1,2-phenylenedioxydiacetamide, 4-octadecanoyloxymethyl-N,N,N',N'-tetracyclohexyl-1,2-phenylenedioxydiacetamide, bis[(12-crown-4)methyl]dodecylmethylmalonate. Exemplary calcium ion ionophores include, for example, bis(didecylphosphate), bis(4-octylphenylphosphate), bis(4-(1,1,3,3-tetramethylbutyl)phenylphosphate tetracosamethylcyclododecasiloxane, N,N'-di(11-ethoxycarbonyl)undecyl)-N,N',4,5-tetramethyl-3,6-dioxaoctane diamide. Barium ion ionophores include, for example, calcium di(2-ethylhexyl)phosphate+decan-1-ol, barium complex of nonylphenoxypoly(ethyleneoxy)ethanol in ortho-nitrodiphenyl ether. Chloride ion ionophores include, for example, {μ-[4,5-dimethyl-3,6-bis(octyloxy)-1,2-phenylene]}bis(trifluoroacetato-O)dimercuri (ETH 9009), {μ-[4,5-dimethyl-3,6-bis(dodecyloxy)-1,2-phenylene]}bis(mercury chloride) (ETH 9033), 5,10,15,20-tetraphenyl-21H,23H-porphin manganese (III) chloride (MnTPPCl), tributyltin chloride (TBTCl) and trioctyltin chloride (TOTCl). Bicarbonate ion ionophores of the invention include, for example, quaternary ammonium ion exchanger p-octodecyloxy-meta-chlorophenyl-hydrazone-mesoxalonitrile. Ammonium ion ionophores include, for example, nonactin and monactin. Nitrate ion ionophores include, for example, tridodecylhexadecylammonium nitrate+n-octyl-ortho-nitrophenyl, 1:10 phenanthroline nickel (II) nitrate+para-nitrocymene. Lithium ion ionophores

include, for example, N, N'-diheptyl-N,N', 5,5-tetramethyl-3,7-dioxononanediamide), 12-crown-4,6,6-dibenzyl-14-crown-4.

A chromoionophore is an ionophore that changes its optical properties in the visible spectrum depending on the state of complexation. Chromoionophores for use in sensors are typically proton-sensitive dyes that change absorbance (and fluorescence in many cases) depending on the degree of protonation, although chromoionophores that change absorbance in response to other ions can also be used. The chromoionophores are preferably highly lipophilic to inhibit leaching from the sensor matrix. Suitable chromoionophores include Chromoionophore I (i.e., 9-(Diethylamino)-5-(octadecanoylimino)-5H-benzo[a]phenoxazine), Chromoionophore II (i.e., 9-Dimethylamino-5-[4-(16-butyl-2,14-dioxo-3,15-dioxaeicosyl)phenylimino]benzo[a]phenoxazine) and Chromoionophore III (i.e., 9-(Diethylamino)-5-[(2-octyldecyl)imino]benzo[a]phenoxazine). Chromoionophore II exhibits light absorbance peaks at 520 nm and 660 nm and a fluorescent emission peak at 660 nm. Chromoionophore III has light absorbance peaks at 500 nm and 650 nm and fluorescent emission peaks at 570 nm and 670 nm.

Quantum dots are fluorescent semiconductor nanocrystals having a characteristic spectral emission, which is tunable to a desired energy by selection of the particle size, size distribution and composition of the semiconductor nanocrystal. The emission spectra of a population of quantum dots have linewidths as narrow as 25-30 nm, depending on the size distribution heterogeneity of the sample population, and lineshapes that are symmetric, gaussian or nearly gaussian with an absence of a tailing region. Advantageously, the range of excitation wavelengths of the quantum dots is broad. Consequently, this allows the simultaneous excitation of varying populations of quantum dots in a system having distinct emission spectra with a single light source, e.g., in the ultraviolet or blue region of the spectrum.

In certain embodiments, quantum dots of the sensor described herein are, for example, inorganic crystallites between 1 nm and about 1000 nm in diameter, preferably between about 2 nm and about 50 nm, more preferably about 5 nm to 20 nm, such as about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nm. Such quantum dots include a "core" of one or more first semiconductor materials, and which may be surrounded by a "shell" of a second semiconductor material. A semiconductor nanocrystal core surrounded by a semiconductor shell is referred to as a "core/shell" semiconductor nanocrystal. The surrounded "shell" will most preferably have a bandgap greater than the bandgap of the core material and can be chosen so to have an atomic spacing close to that of the "core" substrate. The core and/or the shell material can be a semiconductor material including, but not limited to, those of the group II-VI (ZnS, ZnSe, ZnTe, CdS, CdSe, CdTe, HgS, HgSe, HgTe, MgTe and the like) and III-V (GaN, GaP, GaAs, GaSb, InN, InP, InAs, InSb, AlAs, AlP, AlSb, AlS, and the like) and IV (Ge, Si, Pb and the like) materials, and an alloy thereof, or a mixture thereof.

In certain aspects, a sensor comprises exactly one quantum dot. In certain embodiments, a sensor comprises more than one quantum dot, for example, 2, 3, 4, or 5 quantum dots. In certain embodiments wherein the sensor comprises more than one quantum dot, the sensor comprises two or more types of quantum dots, each type having a distinct emission wavelength, e.g., independently selected from, for example, 490, 520, 545, 560, 580, 620, 655 nm. The availability of two distinct wavelength emissions (e.g., one or more quantum dots of wavelength 545 nm and one or more quantum dots with emission wavelength of 655 nm) may allow improvements in recording of changes in ion concentration by using the ratio of the two distinct signals. Fluctuations in fluorescence that are common to both signals should theoretically cancel in a ratio. The detectable fluorescence emission of the quantum dot particles may fluctuate depending on variables including number of quantum dots, quantum dot location within the cell, photobleaching, and possible changes in excitation light intensity, all effects that can occur slowly and are not related to ion presence or concentration. Therefore, effects including number of quantum dots, quantum dot location within the cell, photobleaching, and possible changes in excitation light intensity, may be attenuated.

In certain embodiments, the fluorescence signal of the quantum dot may trigger a detectable event within the cell. For example, fluorescence may in turn excite a secondary dye or quantum dot in the particle that easily generates reactive oxygen species (ROS). The ROS would then attack the cell, effectively stimulating necrosis (cell death), which may then be detected either visually or using markers sensitive to cell death. Alternatively, instead of including a secondary component within the particle, another particle may be added to the cell or cell culture. This additional particle may, for example, comprise a photo-degradable polymer membrane. When the primary sensor fluoresces, the emitted light will rupture the secondary particle, releasing its contents. The contents may, for example, be a drug that is therapeutic or apoptotic, e.g., triggering another detectable event.

The sensor may comprise an additive, e.g., to embed charge sites within the polymer phase and/or to help enforce charge neutrality within the sensor. For sensors targeting cations, the additive can be any inert and preferably lipophilic component that has a negative charge associated with it. For sensors targeting anions, the additive is positively charged and preferably lipophilic. The additive allows the polymer phase to carry a corresponding amount of oppositely charged particles while maintaining overall charge neutrality of the sensor. The concentration ratio of additive to chromoionophore is preferably 1:1, thereby allowing the chromoionphore to become completely protonated or deprotonated. One suitable additive for sensors targeting negative ions is potassium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (KTFPB). The lipophilic, anionic component TFPB molecules are retained by the polymer phase, and the potassium ions are either complexed by the ionophore or expelled into the sample solution through diffusion. In one particular implementation, the sensor film is composed of a suspension produced from about 60 mg of DOS, 30 mg of PVC, and up to about 5 mg of additive, ionophore, and chromoionophore.

In a sample solution, the sensor continuously extracts or expels, for example, analyte cations depending on ion activity in the sample solution. The ion activity of a sample solution can be monitored by observing the fluorescence of a sensor of the invention in the sample solution. As depicted in FIG. **1**, the sensor may fluoresce in the presence of a cationic analyte **21**, and not in the absence of said analyte, Mode A. In such embodiments, the chromoionophore **24**, of the sensor absorbs photons **25**, of a quantum dot **26**, when the cationic analyte **21** is not bound to the ionophore **22**. In such embodiments, the wavelength of photons **25** emitted from the quantum dot **26** when excited with a light source such as UV or visible light fall within the absorbance range, e.g., maximum absorbance range, of the chromoionophore **24** bound to a proton **23**, such that the fluorescence of the quantum dot is attenuated or completely undetectable from outside of the polymer matrix **20** (Mode A, sensor on the left). As the target ion **21** increases in concentration in solution, the ions **21** are drawn into the polymer matrix **20** to bind with the ion-selective ionophore

22. To maintain charge neutrality within the polymer matrix 20, protons 23 dissociate from the chromoionophore 24 in the sensor and diffuse out of the polymer matrix 20 into the sample solution, altering the absorbance properties of the chromoionophore 24. The deprotonated chromoionophore 24 has a shifted absorbance region such that the photons 25 emitted by the quantum dot 26 are no longer absorbed by the chromoionophore 24 (Mode A, sensor on the right). The sensor then emits a detectable signal indicating the presence of the analyte.

In an alternate embodiment for detecting cationic analytes, FIG. 1, Mode B, the quantum dot 27 of the sensor emits photons 25 that are not absorbed by the chromoionophore 24 in the absence of the cationic analyte 21. In certain such embodiments, the chromoionophore 24 absorbs photons 25 of the quantum dot 27 when the cationic analyte 21 is bound to the ionophore 22. In such embodiments, the wavelength of emitted photons 25 from the quantum dot 27 when excited with a light source such as UV or visible light, do not fall within the absorbance range, e.g., the maximum absorbance range, of the chromoionophore 24 when bound to a proton 23, such that the fluorescence of the quantum dot 27 is emitted from the polymer matrix 20 (Mode B, sensor on the left). As the target ion 21 increases in concentration in solution, the ions 21 are drawn into the polymer matrix 20 to bind with the ion-selective ionophore 22. To maintain charge neutrality within the polymer matrix 20 of the sensor, protons 23 dissociate from the chromoionophore 24 of the sensor and diffuse out of the polymer matrix 20 into the sample solution, altering the absorbance properties of the chromoionophore 24. The deprotonated chromoionophore 24 has a shifted absorbance region such that the photons 25 emitted by the quantum dot 27 are absorbed by the chromoionophore 24 (Mode B, sensor on the right). The sensor signal is attenuated or extinguished indicating the presence of the analyte.

In an embodiment for detecting anionic analytes, depicted in FIG. 2, Mode A, the ionophore 22 of the sensor selectively binds an anionic analyte 28 or a group of anionic analytes. In certain such embodiments, the sensor comprises a chromoionophore 24 which absorbs photons 25 emitted from the quantum dot 26 upon excitation, e.g., by light such as UV or visible, when the ionic analyte 28 is not bound to the ionophore 22 of the sensor. In such a state, the wavelengths of the photons 25 emitted by the quantum dot 26 are within the absorbance range, e.g., the maximum absorbance range, of the chromoionophore 24 in a deprotonated state and the fluorescence detected outside of the polymer matrix 20 is attenuated or undetectable from outside the sensor (FIG. 2, Mode A, sensor on the left). As the target ion 28 increases in concentration in the sample solution, the anionic analyte 28 is drawn into the polymer matrix 20, binding with the ion-selective ionophore 22. To maintain charge neutrality within the polymer matrix 20, protons 23 diffuse from the sample solution into the polymer matrix 20, protonating the chromoionophores 24 such that the absorbance properties are altered. The protonated chromoionophore 24 has a shifted absorbance region such that the photons 25 of the quantum dot 26 are not absorbed by the chromoionophore 24 (FIG. 2, Mode A, sensor on the right). The sensor emits a detectable fluorescence signal indicating the presence of the analyte 28.

In an alternate embodiment for detecting anionic analytes, depicted in FIG. 2, Mode B, the ionophore of the sensor selectively binds an anionic analyte 28 or a group of anionic analytes. In certain such embodiments, the sensor comprises a chromoionophore 24 which does not absorb photons 25 emitted from the quantum dot 26, upon excitation, e.g., by light such as UV or visible, when the ionic analyte 28 is not bound to the ionophore 22 of the sensor. In such a state, the wavelengths of the photons 25 emitted by the quantum dot 26 are outside of the absorbance range, e.g., the maximum absorbance range, of the chromoionophore 24 in a deprotonated state and the fluorescence detected outside of the polymer matrix 20 is attenuated or absent (FIG. 2, Mode B, sensor on the left). As the target ion 28 increases in concentration in the sample solution, the anionic analyte 28 is drawn into the polymer matrix 20, binding with the ion-selective ionophore 22. To maintain charge neutrality in the polymer matrix 20, protons 23 diffuse from the sample solution into the polymer matrix 20, protonating the chromoionophores 24 such that the absorbance properties are altered. The protonated chromoionophore 24 has a shifted absorbance region such that the photons 25 of the quantum dot 26 are not absorbed by the chromoionophore 24 (FIG. 2, Mode B, sensor on the right). The sensor signal is attenuated or extinguished indicating the presence of the analyte 28.

In certain embodiments, a fluorescent dye can be used as a source of fluorescence in place of a quantum dot in any of the embodiments described herein. In certain embodiments, a sensor comprises one or more fluorescent dyes.

The following is a non-limiting, illustrative list of target ion (21 or 28)/ionophore 22 pairings suitable for use in the sensors: potassium/Potassium Ionophore III (i.e., BME-44, 2-Dodecyl-2-methyl-1,3-propanediyl bis[N-[5'-nitro(benzo-15-crown-5)-4'-yl]carbamate]), sodium/Sodium Ionophore IV (i.e., 2,3:11,12-Didecalino-16-crown-5 2,6,13,16,19 Pentaoxapentacyclo[18.4.4.4^{7,12}.0^{1,20}.0^{7,12}]dotriacontane), sodium/Sodium Ionophore V (i.e., 4-Octadecanoyloxymethyl-N,N,N',N'-tetracyclohexyl-1,2-phenylenedioxydiacetamide), sodium/Sodium Ionophore VI (i.e., Bis[(12-crown-4)methyl]dodecylmethylmalonate Dodecylmethylmalonic acid bis[(12-crown-4)methyl ester]), sodium/Sodium Ionophore X (4-tert-Butylcalix[4]arene-tetraacetic acid tetraethylester), calcium/Calcium Ionophore III (i.e., Calimycin), and calcium/Calcium ionophore IV (i.e., N,N-Dicyclohexyl-N',N'-dioctadecyl-diglycolic diamide). For target anions, illustrative target ion/ionophore pairings include chloride/Chloride Ionophore III (i.e., 3,6-Didodecyloxy-4,5-dimethyl-o-phenylene-bis(mercury chloride) and nitrite/Nitrite Ionophore I (i.e., Cyanoaqua-cobyrinic acid heptakis(2-phenylethyl ester)).

In certain embodiments, the sensor further comprises a surface modifier (SM). In certain embodiments, the SM comprises a molecule that promotes the delivery or localization of the sensor within a cell. SMs of the invention include molecules with a hydrophilic portion 40 and a hydrophobic portion 42, FIG. 3. In certain embodiments, the hydrophobic portion 42 of the SM anchors the SM to the hydrophobic polymer matrix 41. In certain embodiments, the SM is disposed on the surface of the sensor, e.g., covers a portion of the surface or covers the entire surface. Exemplary hydrophobic portions 42 of the SM include but are not limited to, lipids and hydrophobic polymers. In certain embodiments, the hydrophilic portion 40 of the SM is disposed on the surface of the sensor. An exemplary hydrophilic portion 40 includes, but is not limited to, polyethylene glycol (PEG). In certain embodiments, the hydrophilic portion (PEG) is bound to the hydrophobic portion (lipid) through a linker (e.g., phosphate, ceramide).

In certain embodiments, the sensor further comprises a targeting moiety. In certain embodiments, the targeting moiety is bound to the polymer matrix. In certain embodiments, the targeting moiety is bound to the SM on the surface of the polymer matrix. The targeting moiety, which assists the sensor in localizing to a particular target area, entering a target

cell(s), and/or locating proximal to an ion channel, may be selected on the basis of the particular condition or site to be monitored. The targeting moiety may further comprise any of a number of different chemical entities. In one embodiment, the targeting moiety is a small molecule. Molecules which may be suitable for use as targeting moieties in the present invention include haptens, epitopes, and dsDNA fragments and analogs and derivatives thereof. Such moieties bind specifically to antibodies, fragments or analogs thereof, including mimetics (for haptens and epitopes), and zinc finger proteins (for dsDNA fragments). Nutrients believed to trigger receptor-mediated endocytosis and therefore useful targeting moieties include biotin, folate, riboflavin, carnitine, inositol, lipoic acid, niacin, pantothenic acid, thiamin, pyridoxal, ascorbic acid, and the lipid soluble vitamins A, D, E and K. Another exemplary type of small molecule targeting moiety includes steroidal lipids, such as cholesterol, and steroidal hormones, such as estradiol, testosterone, etc.

In another embodiment, the targeting moiety may comprise a protein. Particular types of proteins may be selected based on known characteristics of the target site or target cells. For example, the probe can be an antibody either monoclonal or polyclonal, where a corresponding antigen is displayed at the target site. In situations wherein a certain receptor is expressed by the target cells, the targeting moiety may comprise a protein or peptidomimetic ligand capable of binding to that receptor. Proteins ligands of known cell surface receptors include low density lipoproteins, transferrin, insulin, fibrinolytic enzymes, anti-HER2, platelet binding proteins such as annexins, and biological response modifiers (including interleukin, interferon, erythropoietin and colony-stimulating factor). A number of monoclonal antibodies that bind to a specific type of cell have been developed, including monoclonal antibodies specific for tumor-associated antigens in humans. Among the many such monoclonal antibodies that may be used are anti-TAC, or other interleukin-2 receptor antibodies; 9.2.27 and NR-ML-05 to the 250 kilodalton human melanoma-associated proteoglycan; and NR-LU-10 to a pancarcinoma glycoprotein. An antibody employed in the present invention may be an intact (whole) molecule, a fragment thereof, or a functional equivalent thereof. Examples of antibody fragments are $F(ab')_2$, Fab', Fab, and F_v fragments, which may be produced by conventional methods or by genetic or protein engineering.

Other preferred targeting moieties include sugars (e.g., glucose, fucose, galactose, mannose) that are recognized by target-specific receptors. For example, instant claimed constructs can be glycosylated with mannose residues (e.g., attached as C-glycosides to a free nitrogen) to yield targeted constructs having higher affinity binding to tumors expressing mannose receptors (e.g., glioblastomas and gangliocytomas), and bacteria, which are also known to express mannose receptors (Bertozzi, C R and M D Bednarski Carbohydrate Research 223:243 (1992); J. Am. Chem. Soc. 114:2242, 5543 (1992)), as well as potentially other infectious agents. Certain cells, such as malignant cells and blood cells (e.g., A, AB, B, etc.) display particular carbohydrates, for which a corresponding lectin may serve as a targeting moiety.

In certain embodiments, the sensor may comprise and internalizing moiety such as a polypeptide or small molecule. In certain embodiments, the sensor may comprise an internalizing polypeptide sequence, such as antepennepedia protein, mastoparan (T. Higashijima et al. (1990) J. Biol. Chem. 265:14176), melittin, bombolittin, delta hemolysin, pardaxin, *Pseudomonas* exotoxin A, clathrin, Diphtheria toxin, C9 complement protein, or a fragment of one of the preceding proteins. In certain embodiments, the internalizing moiety is not the HIV transactivating (Tat) protein. In certain embodiments, the internalizing moiety is bound to one or more of the other elements of the sensor. In one embodiment of the invention, the internalizing moiety serves as the targeting moiety (examples of such targeting moieties included herein). An internalizing moiety is capable of crossing a cellular membrane by, e.g., transcytosis, at a relatively high rate, and thereby promote cellular uptake or endosomal escape of molecules to which they are attached. In certain embodiments, the internalizing moiety crosses the membrane of intra- or extracellular vesicles such as endosomes or lysosomes. In certain such embodiments, sensors comprising internalizing moieties are able to escape endosomal vesicles while sensors that lack internalizing moieties are sequestered from the cellular medium inside such vesicles. In such embodiments, the sensor comprising an internalizing moiety can be situated to monitor analyses in the cytosol of the cell. Certain internalizing polypeptides are also known to localize to the nucleus or other cellular structures. Thus a sensor of the present invention which includes such an internalizing peptide sequence may exhibit increased uptake by target cells relative to sensors that lack such a sequence.

The internalizing polypeptide may be part of the targeting moiety or a separate element of the sensor. In one embodiment of the invention, the internalizing polypeptide serves as the targeting moiety (see examples above of such targeting moieties). In another embodiment, the internalizing polypeptide is covalently linked to one or more of the other elements of the sensor. For example, the internalizing polypeptide can be linked to the targeting moiety; to the polymer matrix; to the surface modifier; to the targeting moiety and to the polymer matrix; or to the surface modifier and the polymer matrix. The preferred location of an internalizing polypeptide in a sensor can be determined, e.g., by conducting in vitro assays using target cells, and detecting the sensor signal that is incorporated into the cells or in specific regions within cells.

In one embodiment, the internalizing peptide is derived from the drosophila antepennepedia protein, or homologs thereof. The 60 amino acid long homeodomain of the homeoprotein antepennepedia has been demonstrated to translocate through biological membranes and can facilitate the translocation of heterologous polypeptides to which it is couples. See for example Derossi et al. (1994) J Biol Chem 269: 10444-10450; Perez et al. (1992) J Cell Sci 102:717-722. Recently, it has been demonstrated that fragments as small as 16 amino acids long of this protein are sufficient to drive internalization. See Derossi et al. (1996) J Biol Chem 271: 18188-18-193. The present invention contemplates a sensor comprising at least a portion of the antepennepedia protein (or homolog thereof) sufficient to increase the transmembrane transport of the sensor, relative to the sensor alone, by a statistically significant amount.

While not wishing to be bound by any particular theory, it is noted that hydrophilic polypeptides may be also be physiologically transported across the membrane barriers by coupling or conjugating a component of the sensor to a transportable peptide which is capable of crossing the membrane by receptor-mediated transcytosis. Suitable internalizing peptides of this type can be generated using all or a portion of, e.g., a histone, insulin, transferrin, basic albumin, prolactin and insulin-like growth factor I (IGF-I), insulin-like growth factor II (IGF-II) or other growth factors. For instance, it has been found that an insulin fragment, showing affinity for the insulin receptor on capillary cells, and being less effective than insulin in blood sugar reduction, is capable of transmembrane transport by receptor-mediated transcytosis. Preferred growth factor-derived internalizing peptides include EGF

(epidermal growth factor)-derived peptides, such as CMHIESLDSYTC (SEQ ID NO: 2) and CMYIEALDKYAC (SEQ ID NO: 3); TGF-beta (transforming growth factor beta)-derived peptides; peptides derived from PDGF (platelet-derived growth factor) or PDGF-2; peptides derived from IGF-I (insulin-like growth factor) or IGF-II; and FGF (fibroblast growth factor)-derived peptides. Hydrophilic polypeptides can be bound to a component of the sensor, or they can constitute the targeting moiety.

Another class of translocating/internalizing peptides exhibits pH-dependent membrane binding. For an internalizing peptide that assumes a helical conformation at an acidic pH, the internalizing peptide acquires the property of amphiphilicity, e.g., it has both hydrophobic and hydrophilic interfaces. More specifically, within a pH range of approximately 5.0-5.5, an internalizing peptide forms an alpha-helical, amphiphilic structure that facilitates insertion of the moiety into a target membrane. An alpha-helix-inducing acidic pH environment may be found, for example, in the low pH environment present within cellular endosomes. Such internalizing peptides can be used to facilitate transport of sensors, taken up by an endocytic mechanism, from endosomal compartments to the cytoplasm.

A preferred pH-dependent membrane-binding internalizing peptide includes a high percentage of helix-forming residues, such as glutamate, methionine, alanine and leucine. In addition, a preferred internalizing peptide sequence includes ionizable residues having pKa's within the range of pH 5-7, so that a sufficient uncharged membrane-binding domain will be present within the peptide at pH 5 to allow insertion into the target cell membrane.

A particularly preferred pH-dependent membrane-binding internalizing peptide in this regard is aa1-aa2-aa3-EAALA (EALA)4-EALEALAA-amide (SEQ ID NO: 4), which represents a modification of the peptide sequence of Subbarao et al. (Biochemistry 26:2964 (1987)). Within this peptide sequence, the first amino acid residue (aa1) is preferably a unique residue, such as cysteine or lysine, that facilitates chemical conjugation of the internalizing peptide to a targeting protein conjugate. Amino acid residues 2-3 may be selected to modulate the affinity of the internalizing peptide for different membranes. For instance, if both residues 2 and 3 are lys or arg, the internalizing peptide will have the capacity to bind to membranes or patches of lipids having a negative surface charge. If residues 2-3 are neutral amino acids, the internalizing peptide will insert into neutral membranes.

Yet other preferred internalizing peptides include peptides of apo-lipoprotein A-1 and B; peptide toxins, such as melittin, bombolittin, delta hemolysin and the pardaxins; antibiotic peptides, such as alamethicin; peptide hormones, such as calcitonin, corticotrophin releasing factor, beta endorphin, glucagon, parathyroid hormone, pancreatic polypeptide; and peptides corresponding to signal sequences of numerous secreted proteins. In addition, exemplary internalizing peptides may be modified through attachment of substituents that enhance the alpha-helical character of the internalizing peptide at acidic pH.

Yet another class of internalizing peptides suitable for use within the present invention include hydrophobic domains that are "hidden" at physiological pH, but are exposed in the low pH environment of the target cell endosome. Upon pH-induced unfolding and exposure of the hydrophobic domain, the moiety binds to lipid bilayers and effects translocation of a covalently linked sensor into the cell cytoplasm. Such internalizing peptides may be modeled after sequences identified in, e.g., *Pseudomonas* exotoxin A, clathrin, or Diphtheria toxin.

Pore-forming proteins or peptides may also serve as internalizing peptides herein. Pore forming proteins or peptides may be obtained or derived from, for example, C9 complement protein, cytolytic T-cell molecules or NK-cell molecules. These moieties are capable of forming ring-like structures in membranes, thereby allowing transport of attached sensors through the membrane and into the cell interior.

Mere membrane intercalation of an internalizing peptide may be sufficient for translocation of a sensor across cell membranes. However, translocation may be improved by attaching to the internalizing peptide a substrate for intracellular enzymes (i.e., an "accessory peptide"). It is preferred that an accessory peptide be attached to a portion(s) of the internalizing peptide that protrudes through the cell membrane to the cytoplasmic face. The accessory peptide may be advantageously attached to one terminus of a translocating/internalizing moiety or anchoring peptide. An accessory moiety of the present invention may contain one or more amino acid residues. In one embodiment, an accessory moiety may provide a substrate for cellular phosphorylation (for instance, the accessory peptide may contain a tyrosine residue).

An exemplary accessory moiety in this regard would be a peptide substrate for N-myristoyl transferase, such as GNAAAARR (SEQ ID NO: 5) (Eubanks et al. (1988) Peptides. Chemistry and Biology, Garland Marshall (ed.), ESCOM, Leiden 566-69). In this construct, an internalizing, peptide would be attached to the C-terminus of the accessory peptide, since the N-terminal glycine is critical for the accessory moiety's activity. This hybrid peptide, upon attachment to a sensor component is N-myristylated and will be translocated across the cell membrane.

In certain embodiments the internalizing moiety comprises an amine group. In certain such embodiments, an amine group is bound to any component of the sensor such as the polymer matrix, the surface modifier, the targeting moiety or any combination thereof. The amine group may have advantages in increasing release of nanosensors into the cytosol. One of the unique characteristics of endocytosis that previous studies and methods have exploited is the gradual decrease in compartment pH as the endosome passes from early stage to late stage and eventually forms a lysosome. The amine group is capable of exploiting this phenomenon by protonating as the pH decreases. When the pH of the endosome drops to around 5 most of the amine groups will be protonated. This charge may be sufficient enough to lyse the membrane of the endosome and release the components.

The film of the sensor can be produced in various ways. In one implementation, as described above, a predetermined amount of the sensor mixture (e.g., the combined polymer phase, ionophore, quantum dots/dye, additive, and chromoionophore) is dissolved in a solvent, such as THF. The solution is then deposited, sprayed, or spun onto a surface. The solvent evaporates, leaving the sensor film on the surface.

In another implementation, the film is formed from a deposition of sensor microspheres. To produce the microspheres, a sensor emulsion is formed by injecting a sensor suspension dissolved in THF (e.g., 16 mL THF/100 mg PVC) into a pH buffered solution. The sensor suspension includes approximately 60 mg of DOS, 30 mg of PVC, and up to approximately 5 mg of chromoionophore, additive, and ionophore. The emulsion is then submerged in a sonicating water bath. Typically, 50 μL of the sensor suspension/THF solution is injected into 1,000-1,500 μL of buffered solution. The resulting emulsion contains a mixture of spherical sensor particles ranging in size from 200 nm to 20 pm in diameter. In certain embodiments, the nanosensors range in size from about 5 nm to about 300 nm in diameter, such as about 20 nm to about 200

nm in diameter, e.g., about 100 nm. In certain embodiments, the nanosensors that comprise only one quantum dot range in size from about 5 nm to about 50 nm in diameter, such as about 5 nm to about 25 nm in diameter, e.g., 20 nm. In certain embodiments wherein the particles are non-spherical, the diameter is measured at the widest dimension of the nanosensor. Particles of larger dimension are, of course, readily prepared.

Sensor materials as discussed herein can be sized and shaped in any suitable configuration that can be achieved using the polymer. For example, in certain embodiments, the nanosensors are non-spherical, such as a disk or a cube, or even sculpted or molded into a utilitarian or aesthetic shape. A sensor emulsion can be spun, sprayed, or evaporated onto any surface to create a porous sensor membrane. In certain embodiments, the sensor film can be of a size suitable for the application, such as the coating of a glass slide, the bottoms of wells of a 96-well plate, or even a beverage dispenser, such as a pitcher, tank, or bottle. Films formed from microspheres tend to expose a greater surface area of sensor to a given sample, yielding improved performance characteristics.

In certain aspects, a film of the sensor particles is deposited on the surface of a support. In certain embodiments, the support is an instrument that can be placed in a solution such as a glass rod, a stirring bar, a straw, or glass beads. In certain embodiments, the support is a container in which the ionic solution to be evaluated can be contained. In certain embodiments, the surface of the support is partially coated with the sensor particles while in other embodiments, the support surface is entirely coated with the sensor particles. In certain embodiments, the sensors are incorporated within the support and the support is sculpted into a desired shape such as a stir bar, a film, or a bead.

In certain embodiments, the sensors are used to detect ions in water or other aqueous solutions. In certain embodiments, the support deposited with the sensor particles is used to detect the presence of ions in an aqueous solution. In certain exemplary embodiments, the sensors are used to detect ions in water, e.g., tap water or ground water, to determine the levels of toxic ions in solution or to determine the hardness of the aqueous solution. In certain exemplary embodiments, the sensors are added to manufacturing solutions to measure ions during production of, e.g., the mass production of soda, ion-restoring beverages or other ionic drinks. In certain embodiments, the sensors are used in the laboratory to monitor the ion content of a reaction mixture or stock solution.

In certain embodiments, the sensors are placed in contact with cells in biological samples such as tissues outside of the host specimen. In certain embodiments, the sensors are introduced to cells within a host specimen such as a plant or animal. The nanosensor particles may be introduced into the cells in any suitable manner. In one method, the particles are introduced into a buffer liquid deposited in the biological sample holder. A voltage source then generates a voltage sufficiently strong to electroporate the cells, thereby allowing the nanosensor particles to enter directly into the cells. In another approach, the surfaces of the nanosensor particles are first coated with a substance such as a surface modifier, a targeting moiety, an internalizing moiety or any combination thereof, which assist the particles in crossing through lipophilic membranes. The nanosensor particles contact the cells which bring the particles into their interior in vesicles via endocytosis, pinocytosis, phagocytosis, or similar biological processes. In certain embodiments, the internalizing moiety of the nanosensor particle breaks down the vesicle membrane, releasing the nanosensor particle into the cell cytoplasm. In still other approaches, the particles may be introduced into cells using a glass needle or through ballistic bombardment.

To determine compartmentalization of nanosensors within the cells TEM and fluorescence staining can be used. TEM can be used to determine location of the nanosensor in a cell, may provide a good understanding of nanosensor transport in the cell and serve as a validation of the co-localization staining. The second method, co-localization staining, can be used to determine endosomal release.

Dyes suitable for performing co-localization studies include: FM1-43, FM4-64, Fluorescein, Transferrin, and Lysotracker Red. FM1-43 is a lipophilic dye that readily stains cell membranes. Previous studies have shown the effectiveness of FM1-43 to stain endosomes. Its fluorescence emission is typically greatly increased upon incorporation into a hydrophobic environment. FM1-43 will typically stain the plasma membrane of a cell and remain associated with the lipid bilayer as it forms an endosome. Dye that is not taken into the cell and remains on the plasma membrane can be easily removed by gentle washing. FM4-64 is an analog of FM1-43 and behaves in a very similar fashion. It is more hydrophobic then FM1-43 and therefore may be more suitable for endocytosis studies. FM4-64 has been well characterized as an endosomal stain. The long wavelength emission of FM4-64 may be advantageous when using sensors of different spectral properties similar to the other fluorescent stains being utilized.

In some embodiments, the sensor is attached to the exterior of a cell rather than introduced into the interior. If, for example, the activity of an ion channel is to be studied, the sensor may be attached to the cell surface or placed in close proximity to the cell surface in a location where ion concentrations are in flux, such as adjacent to an ion channel. The sensor may be positioned adjacent to the ion channel of a cell, for example, by covalently linking one or more antibodies that selectively bind the ion channel of interest to a sensor particle as described above. The antibody-linked sensor particles may be added to a cell suspension to bind to the ion channel. This approach can be used to link ion-specific sensors to any feature on the exterior of the cell membrane to which antibodies selectively bind. Alternatively, the sensors may be attached to the cell membrane by other suitable coupling chemistries, e.g., biotin-(strept)avidin complexing or polysaccharide binding. See the thesis "High Throughput Optical Sensor Arrays for Drug Screening" by Daniel I. Harjes (2006), available from the Massachusetts Institute of Technology and incorporated herein by reference.

In certain embodiments, cells or tissues are contacted with both nanosensor particles and a sensor film. In certain embodiments, the quantum dots used in the sensor film differ from the quantum dots used in the nanosensor particles. In particular, the different quantum dots desirably have distinguishable fluorescence characteristics such that an analysis module analyzing the output of a light sensor monitoring the sensor arrangement can differentiate between the output of the sensor film and the nanosensor particles. As a result, the analysis module can differentiate between intracellular target ion concentration and extracellular target ion concentration. In an exemplary embodiment, the sensor film comprises quantum dots of a selected fluorescence wavelength, e.g., 560 nm, and the nanosensor particles comprise quantum dots of a selected fluorescence wavelength, e.g., 655 nm. In addition, the sensor film may include ionophores different from those included in the sensor particles, e.g., nanosensor particles comprising sodium ionophores and sensor films comprising

potassium ionophores. Thus, the sensor arrangement can monitor the concentrations of two different target ions.

In still another embodiment, the sensor film is coated onto the inner surface of a biological sample holder. And in another approach, to accommodate multiwell plates, such as the 96-well plate format often used in assays, one embodiment of the present invention utilizes round glass coverslips coated with the sensor film along with the cells to be monitored. In certain embodiments, larger multiwell plates such as 384- and 1536-well plates are applied with a layer of sensor film disposed on a surface of some or all of the wells. In these embodiments, each well contains a single sensor type to track a specific species of interest; the various sensor types may differ in the ionophore employed and utilize quantum dots with fluorescence wavelengths that are the same or similar. The compound of interest is then added directly to the well. The multiwell plate is then placed in a fluorometer and the fluorescence intensity is monitored with time.

In a typical implementation, a plurality of biological sample holders holding biological samples is provided. Biological samples introduced into the holders may include cells suspended in a buffer solution, but alternatively cells may be adhered to the walls of the biological sample holders. Next, sensors are introduced into biological sample holders and/or are introduced into the cells themselves. Alternatively, the sensors can coat the walls of the biological sample holders. As described above, nanosensor particles can be introduced either by electroporating the cells via electrodes positioned in the biological sample holders or by the chemistry applied to the nanosensor particles breaching vesicle membranes within the cells. Similarly, the sensors can be introduced into the cells using pico-injection, bead loading, a gene gun, or through liposomal delivery techniques known in the art.

An agent, such as a therapeutic, toxin, biological macromolecule (such as a nucleic acid, an antibody, a protein or portion thereof, e.g., a peptide), small molecule (of 2000 amu or less, 1000 amu or less or 500 amu or less), protein, virus, bacteria, chemical compound, mixture of chemical compounds, or an extract made from biological materials such as bacteria, plants, fungi, or animal (particularly mammalian) cells or tissues, or other biologically active agent may be introduced into one or more of the biological sample holders. In one particular implementation using an array of biological sample holders, no agent is introduced into a first row of biological sample holders to preserve a control. A first agent is introduced into a second row of biological sample holders. Additional agents are added to additional rows of the array of biological sample holders. The fluorescence of the sensors introduced into the biological sample holders may be monitored. The monitoring preferably begins prior to introduction of the agents and continues thereafter. Changes in ion concentration resulting from the introduced agents are then determined. By comparing the changes in ion concentration after adding an agent, one can determine the effect of the agent on the cells being tested.

The sensors of the invention can be used to monitor the effects of pharmaceutical agents on biological systems such as the cardiovascular system or the circulatory system. Action potentials generated by cardiac or neural cells in culture are defined by a flux of sodium and potassium into and out of the cell. In certain embodiments, the sensors of the invention measure this ion flux in cardiac cells accurately and spatially in a high throughput manner.

In certain aspects, the sensors are used in the drug discovery process. In certain such embodiments, the sensors are used to measure the efficacy of a therapy. For example, ion-selective sensors may be employed to monitor the effect of ion channel-modulating drugs. In alternative embodiments, sensors are used to screen for cytotoxic substances by, for example, determining ionic flux in cardiac cells in response to a cytotoxic agent and using these values as a comparison for testing novel therapeutic agents.

In certain aspects, the sensors of the invention are implanted into small animals to monitor biological responses to new therapeutic agents. In certain embodiments, the implantable sensors are used to study the mechanism of disease in small animals. In certain such embodiments, the animals, such as rats or mice, are, for example, infected with a disease and the biological functions are monitored by detecting the signal of the implanted optical sensors. In such embodiments, the animal is placed within a monitoring element, e.g., a fluorescent monitoring cell similar to a monitoring element used to take X-rays of small animals, wherein the quantum dots of the sensors are excited, e.g., with UV light, and fluorescence emitted from the sensors within the animal may be detected.

In various embodiments the invention may be constructed to directly detect the presence of particular ions. As illustrated in the tables below, it is known in the art that certain diseases affect particular ion channels in a cell. Accordingly, assays for those ions utilizing the present invention may furnish a diagnostic tool to determine the presence of particular diseases. Accordingly, the scope of the present invention should be understood to also include the application of the heretofore-described subject matter to measure the ions set forth in the following tables, as well as their application to diagnose the presence of the associated diseases also appearing in the following tables.

Channel	Gene	Channel-forming unit/ligand	OMIM	Disease
Cation channels:				
CHRNA1/ ACHRA	CHRNA1	α, ACh	100690	Myasthenia congenita
CHRNA4	CHRNA4	α, ACh	118504	Autosomal dominant nocturnal frontal lobe epilepsy
CHRNB2	CHRNB2	β, ACh	118507	Autosomal dominant nocturnal frontal lobe epilepsy
Polycystin-2	PKD2	α	173910	Autosomal dominant polycystic kidney disease (ADPKD)
CNGA3	CNGA3	α, cGMP	60053	Achromatopsia 2 (color blindness)
CNGB1	CNGB1	β, cGMP	600724	Autosomal recessive retinitis pigmentosa

-continued

Channel	Gene	Channel-forming unit/ligand	OMIM	Disease
CNGB3	CNGB3	β, cGMP	605080	Achromatopsia 3
Sodium channels:				
Na. 1.1	SCN1A	α	182389	Generalized epilepsy with febrile seizures (GEFS+)
Na. 1.2	SCN2A	α	182390	Generalized epilepsy with febrile and afebrile seizures)
Na. 1.4	SCN4A	α	603967	Paramyotonia congenital, potassium aggressive myotonia, hyperkalemic periodic paralysis
Na. 1.5	SCN5a	α	600163	Long-QT syndrome, progressive familial heart block type 1, Brugada syndrome (idiopathic ventricular arrhythmia)
SCNIB	SCNIB	β	600235	Generalized epilepsy with febrile seizures (GEFS+)
ENACα	SCNNIA	α	600228	Pseudohypoaldosteronism type 1 (PHA1)
ENaCβ	SCNN1B	β	600760	PHA1, Liddle syndrome (dominant hypertension
ENaCγ	SCNN1G	γ	600761	PHA1, Liddle syndrome
Potassium channels:				
K, 1.1.	KCNA1	α	176260	Episodic ataxia with myokymia
KCNQI/K, LQT1	KCNQ1	α	192500	Autosomal dominant long-QT syndrome (Romano-Ward) Autosomal recessive long-QT syndrome with deafness (Jervell-Lange-Nielsen)
KCNQ2	KCNQ2	α	602235	BFNC (epilepsy), also with myokymia
KCNQ3	KCNQ3	α	602232	BFNC (epilepsy)
KCNQ4	KCNQ4	α	603537	DFNA2 (dominant hearing loss)
HERG/KCNH2	KCNH2	α	152427	Long-QT syndrome
Kir1. 1/ROMK	KCNJ1	α	600359	Bartter syndrome (renal salt loss, hypokalemic alkalosis)
Kir2. 1/IRK/ KCNJ2	KCNJ2	α	600681	Long-QT syndrome with dysmorphic features (Andersen syndrome)
Kir6.2/ $KATATP_{ATP}$	KCNJ11	α	600937	Persistent hyperinsulinemic hypoglycemia of infancy (PHHI)
SURI	SURI	β	600509	PHHI
KCNE1/Mink/ ISK	KCNE1	β	176261	Autosomal dominant long-QT syndrome (Romano-Ward) Autosomal recessive long-QT syndrome with deafness (Jervell-Lange-Nielson)
KCNE2/MiRP1	KCNE2	β	603796	Long-QT syndrome
KCNE3/MiRP2	KCNE3	β	604433	Periodic paralysis
Calcium channels:				
Ca. 1.1	CACNA1S	α	114208	Hypokalemic periodic paralysis, malignant hyperthermia
Ca, 1.4	CACNA1F	α	300110	X-linked congenital stationary night blindness
Ca, 2.1	CACNA1A	α	601011	Familial hemiplegic migraine, episodic staxia, spinocerebella ataxia type 6
RyRI	RYRI	α	180901	Malignant hyperthermia, central core disease
RyR2	RYR2	α	180902	Catecholaminergic polymorphic ventricular tachycardia, arrhythmogenic right ventricular dysplasia type 2
Chloride channels:				
CFTR	ABCC7	α	602421	Cystic fibrosis, congenital bilateral asplasia of vas deference

-continued

Channel	Gene	Channel-forming unit/ligand	OMIM	Disease
CIC-1	CLCN1	α	118425	Autosomal recessive (Becker) or dominant (Thomsen myotonia
CIC-5	CLCN5	α	300008	Dent's disease (X-linked proteinuria and kidney stones)
CIC-7	CLCN7	α	602727	Osteopetrosis (recessive or dominant)
CIC-Kb	CLCNKB	α	602023	Bartter syndrome type III
Barttin	BSND	β	606412	Bartter syndrome type IV (associated with sensorineural deafness)
GLRA1	GLRA1	α, glycine	138491	Hyperekplexin (startle disease)
GABAα1	GABRA1	α GABA	137160	Juvenile myoclonus epilepsy
GABAγ2	GABRG2	γ, GABA	137164	Epilepsy
Gap junction channels:				
Cx26	GJB2		121011	DFNB3 (autosomal dominant hearing loss) DFNB1 (autosomal recessive hearing loss)
Cx30	GJB4		605425	DFNA3
Cx31	GJB3		603324	DFNA2
Cx32	GJB1		304040	CMTX (X-linked Charcot-Mari-Tooth neuropathy)
AChR α7				Inflammation
ClC7				Osteoporosis
Ether-a-go-go (eag, erg, elk)				Cancer
Gardos channel				Sickle cell anemia
P2X7				Immune disorders
TRPC6				Asthma, COPD
TRPM1				Melanoma
TRPM2				Asthma
TRPM4				Immune disorders
TRPM7				Stroke
TRPM8				Prostate cancer
TRPV1				Urinary incontinence, pain

The third column classifies channel proteins into α, β, and γ subunits, where α subunits are always directly involved in pore formation, Several β subunits are only accessory (i.e., do not form pores), as is the case, for example, with SCN1B and barttin. Others (e.g. of ENaC and GABA receptors) participate in pore formation. For ligand-gated channels, the ligand is given. Note that GABA and glycine act from the extracellular side, whereas cGMP is an intracellular messenger.

Gene	Accession ID	Gene Locus	Sodium Channel Type/Disease	Tissue Expression
SCN1A	GDB: 118870 S71446	2q24	SCN1, vg type 1, α-subunit (280 KDa)	Brain
SCN1B	GDB: 127281 U12188-12104 L16242, L10338	19q13.1	Hs.89634, vg type 1 β_1 subunit (38 KDa)	Brain, heart, skeletal muscle
SCN2A1	GDB: 120367	2q23	SCN2A, HBSC1, vg type II, α_1 - subunit (280 KDa)	Brain, peripheral nerve
SCN2A2	CDB: 133727	2q23-24.1	HBSCH, vg type II, α_2 - subunit vg type II, β_2 - subunit (33 KDa)	Brain
SCN2B	GDB: 118871 AF019498			
SCN3A	GDB: 132151 S69887	2q24-31	vg type III, α-subunit (280 kDa)	Brain
SCN4A	GDB: 125181 L04216-L04236	17q23.1-25.3	SkM1, vg type IV α-subunit (260 kDa), hyperkalemic periodic paralysis, paramyotonia congentia, potassturn-aggravated myotonia	Skeletal muscle
SCN4B	GDB: 125182	3q21	vg type IV, β-subunit,	Heart, fetal skeletal

-continued

Gene	Accession ID	Gene Locus	Sodium Channel Type/Disease	Tissue Expression
SCN5A	GDB: 132152		SkM2, hH1, vg type V, α-subunit, long Q-T syndrome 3	muscle
SCN6A	GDB: 132153	2q21-23	Hs99945, vg type VI, α-subunit	Heart, uterus, fetal and denervated skeletal muscle
SCN7A	GDB: 228137	12q13	vg type VII, α-subunit	Brain, spinal cord
SCN8A	GDB: 631695		vg type VIII, α-subunit, motor end-plate disease + ataxia in mice	
SCN9A	GDB: 3750013		vg type IX, α-subunit neuroendocrine type	Thyroid and adrenal gland
SCN10A	GDB: 750014	1pter-p36.3	hPN3, vg type X	Sensory neurons, dorsal root ganglia
SCNN1A	GDB: 366596 Z92978	12pt3	SCNN1, nvg type 1 α-subunit of ENaC	Kidney, lung, colon
SCNN1B	GDB: 434471	16p12.2-p12.1	nvg 1 β-subunit, Liddle's syndrome, pseudohypoaldosterontsm I	Kidney, lung, colon
SCNN1D	GDB: 6053678	1p36.3-p36.2	DnaCh, nvg 1 δ-subunit	Kidney, lung, colon
SCNN1G	GDB: 568769 X87160 U53835-53853	16p122-p12.1	nvg 1 γ-subunit, Liddle's syndrome, pseudohypoaldosterontsm I	Kidney, lung, colon
CACNA1A CACNL1A4	GDB: 126432 Z80114-Z80155, X99697, U79666	19p13 19p13.1	P/Q type α_{1A}-subunit, eqisodic ataxia 2, familial hemiplegic migraine, spinocerebellar ataxia 6; tottering, leaner, and rolling mice	Brain (cortex, bulbus, olfacorius, hippocampus, cerebellum, brain stem), motoneurons, kidney
CACNA1B CACNL1A5	GDB: 580689 M94172, M94173	9q34	CACNN, N-type α_{1A}-subunit	Central, peripheral nervous system
CACNA1C CACNL1A1	GDB: 126094 L29636, L29634, L29629	12p13 12p13.3	CCHL1A1, L-type α_{1A}-subunit	Heart, fibroblasts, lung, smooth muscle (2 splice variants)
CACNA1D CACNL1A2	GDB: 128872	3p14.3 3p21.3.2?	CCHL1A2, L-type α_{1D}-subunit	Brain, pancreas, neuroendocrine
CACNA1E CACNL1A6	GDB: 434408	1q25-31	R-type α_{1C}-subunit	Brain, skeletal muscle (end plate)
CACNA1F	GDB: 6053864	Xp11.23-11.22	α_{1F}-Subunit	Retina
CACN1AG	AF27964	17q22	T-type α_{1G}-subunit	Brain
CACNA1S CACNL1A8	GDB: 126431 Z22672, L33798 U30666-U30707	1q31-32	L-type α_{1B}-subunit (5% 212, 95% 190 kDa), malignant hyperthermia 5, hypokalemic periodic paralysis	Skeletal muscle (brain, kidney)
CACNA2 CACNL2A	GDB: 132010 Z28613, Z28609 Z28605, Z28602 Z28699, M76559	7q21-22	CACNA2, CACNA2D1, α_{g} g-subunit (175 kDa), MHS3	α_{2A}; skeletal muscle, heart, brain, ileum; α_{2B}; brain; α_{2CVD} aorta
CACNB1 CACNLB1	GDB: 132012 GDB: 1073281 U86952-U86961 M76560, L06111 GDB: 193328	17q21-22	β_1-Subunit (524 aa, 54 kDa)	β_1A/M; skeletal muscle β_1B/C; brain, heart, spleen
CACNB2 CACNLB2	GDB: 132014 Q08289	10p12	MYSB, β_2-subunit	β_2-A/B/E; brain, heart, lung, aorta
CACNB3	GDB:	12q13	β_2-subunit (482 aa)	Brain, heart, lung,

-continued

Gene	Accession ID	Gene Locus	Sodium Channel Type/Disease	Tissue Expression
CACNLB3	341023 L27584			spleen, skeletal and smooth muscle, aorta, trachea, ovary, colon
CACNB4	GDB: 6028693	2q22-23	β_2-subunit, lethargic mice	Brain, kidney
CACNG CACNLG	GDB: 132015 L07738	17q24	γ-Subunit (222 aa, 30 kDa)	Skeletal muscle, lung
CACNG2			γ2-Subunit, stargazin, absence epilepsy stargazer, waggler mice	Brain
RYR1	GDB: 120359	19q13.1	Ryanodine receptor 1, Ca release channel, 3 splice variants, malignant hyperthermia 1, central core disease	Skeletal muscle, testis, brain, submaxillary and adrenal glands, spleen
RYR2	GDB: 125278	1pter-qter 1q42.1-43	RYR2, calcium release channel	Heart, smooth muscle
RYR3	GDB: 138451	15q14 15q14-15	RYR3, calcium release channel	Brain, neonatal skeletal muscle, adult diaphragm
KCNA1	GDB: 127903 LO2750	12p13	RBK1, HUK1, MBK1, AEMK, Kv1.1, Shaker homolog 1, Shaker, episodic ataxia 1 (with myokymia)	Brain, nerve, heart, skeletal muscle, retina, pancreatic islet
KCNA1B		3q26.1	Kvβ1.1, Kvβ1.3 (splice product), β-subunit	
KCNA2	GDB: 128062 X17622	12pter-qter	HK4, Kv1.2, Shaker homolog 2	Brain, nerve, heart, pancreatic islet
KCNA2B		1p36.3	Kvβ1.2, β-subunit	
KCNA8	GDB: 128079 L23499	1p13.3	Hs.1750, MK3, HLK3, HPCN3, Kv1.3, Shaker homolog 3	Skeletal muscle, lymphocytes (brain, lung, thymus, spleen)
KCNA4	GDB: 126730 M60450 M55514	11p14	Hs.89647, Hs.1854, HK1, HPCN2, Kv1.4, Shaker homolog 4	Brain, nerve, heart, fetal skeletal muscle, pancreatic islet
KCNA4L	GDB: 386059	11q14	Shaker homolog type 4-like	
KCNA5	GDB: 127904 M83254 M60451	12p13.3-13.2 12p13 12p13.33-12.31	Hs.89509, HK2, HPCNI, Kv1.5 Shaker homolog 5	Brain, heart, kidney, lung, skeletal muscle, pancreatic islet
KCNA6	GDB: 128080 X17622	12p13	HBK2, Kv1.6, Shaker homolog 6	Brain, pancreatic islet
KCNA7	GDB: 127905	19q13.3	HAK6, Kv1.7 Shaker homolog 7	
KCNA8			see KCNQ1	
KCNA9			see KCNQ1	
KCNA10	GDB: 5885822		Shaker homolog type 10, cGMP activated	
KCNB1	GDB: 128081	20q13.2	Kv2.1, Shab homolog 1	Brain, heart, kidney, retina, skeletal muscle
KCNB2			Kv2.2, Shab homolog 2	Brain, heart, retina
KCNC1	GDB: 128082 S56770 M96747	11p15.1	Kv3.1, Shaw homolog 1	Brain, skeletal muscle, spleen, lymphocytes
KCNC2	GDB: 127906	19q13.3-13.4	Kv3.2, Shaw homolog 2	Brain
KCNC3	GDB: 127907	19q13.3	Kv3.3, Shaw homolog 3	Brain, liver
KCNC4	GDB: 127908	1p21	Kv3.4, HKSHIIIC, Shaw homolog 4	Brain, skeletal muscle
KCND1	GDB: 128083		Kv4.1, Shal homolog 1	Brain
KCND2	GDB: 134771		RK5, Kv4.2, Shal homolog 2	Brain, heart, aorta
KCND3	GDB: 134772		Kv4.3, KSHIVB, Shal homolog 3	
KCNE1	GDB: 127909	21q22.1-22.2	MinK, ISK, vg Isk homolog 1 (129 aa), long Q-T syndrome 5	Kidney, submandibular gland, uterus, heart, cochlea, retina

-continued

Gene	Accession ID	Gene Locus	Sodium Channel Type/Disease	Tissue Expression
KCNMA1	GDB: 386031 U09383-4 U02632	10pter-qter 7q32.1	SLO, Hs.62679, α-subunit member 1, α-subunit of maxiK or BK channel	Fetal skeletal muscle
KCNMB1	GDB: 6099615 U42600	5q34	hSLO-β, β-subunit member 1 (191 aa), β-subunit of max IK or BK channel	Smooth, fetal skeletal muscle, brain (hippocampus, corpus callosum)
KCNN1	U69883		SK(Ca)1, small-conductance Ca-activated K channel, apamin-insensitive	Brain, heart
KCNN2			SK(Ca)2, apamin sensitive	Brain, adrenal gland
KCNN3	Y08263 AA285078	1q?	SK(Ca)3, small-conductance Ca-activated K channel, intermediate apamin sensitivity	Brain, heart, (human embryonic) skeletal muscle, liver
KCNN4	AF022150 AF022797 AF033021 AF000972	19q13.2	IK1, intermediate-conductance Ca-activated K channel, KCa4, SK4, Gantos channel	T lymphocytes, colon, smooth muscles, prostata, red blood cells, neurons
KCNQ1	GDB: 741244 U40990	11p15.5	KCNA9, (KV)LQT1, KQT-like subfamily member 1, long Q-T syndrome 1	Heart, cochlea, kidney, lung, placenta, colon
KCNQ2	GDB: 9787229, Y15065, AF033348	20q13.3	KQT-like subfamily member 2 (872 aa)	Brain
KCNQ3	GDB: 9787230 AF033347	8q24.22-24.3	KQT-like subfamily member 3 (825 aa)	Brain
HERG	GDB: 407638	7q35-36	HERG, similar to ether-a-go go (eag), Ikr, long Q-T syndrome 2	Brain, heart
KCNJ1	GDB: 204206 U65406, U12541	11q24	ROMK1, Kir1.1, Hs.463, Bartter/hyperprostaglandin E syndrome	Kidney, pancreatic islets
KCNJ2	GDB: 278964 U12507	17pter-qter	IRK1, Kir2.1, Hs.1547	Muscle, neural tissue, heart
KCNJ3	GDB: 278325 U50964	2q24.1	GIRK1, Kir3.1	Heart, cerebellum
KCNJ4	GDB: 374080 Z97056	22q13.1	HIR, HIRK1, HIRK2, Kir2.3	Heart, skeletal muscle, brain
KCNJ5	GDB: 547948	11q24	CIR. KATP1, GIRK4, Kir3.4	Heart, pancreas
KCNJ6	GDB: 547949 U24660	21q22.1	KCNJ7, GIRK2, KATP2, BIR1, Kir3.2, ataxia, weaver mice	Cerebellum, pancreatic islet
KCNJ8	GDB: 633096	12p11.23]	Kir6.1, uKATP, ubiquitous K_{ATP} α-subunit	Brain, heart, skeletal, smooth muscle, others
KCNJ10	GDB: 3750203	1q22-23]	Kir1.2, Kir4.1	Glia
KCNJ11	GDB: 7009893	[11p15.1]	Kir6.2, BIR, K(ATP) α-subunit, hyperinsulinemic hypoglycemia	Pancreatic islets
KCNJ12	GDB: 4583927	[17p11.1]	Kir2.2	
KCNJ15	GDB: 6275865	[21q22.2]	Kir4.2	
KCNJN1	GDB: 6108062	[ ]	Kir2.2v, subfamily inhibitor 1	
SUR1	GDB: 591970	[11p15.1]	SUR(1), sulfonylurea receptor, K(ATP) β-subunit, hyperinsulinemic hypoglycemia	Pancreatic islets
SUR2		12p12.1]	SUR2, SUR2A, B, sulfonylurea receptor 2 (1545-aa), β-subunit of K(ATP)	2A: heart, 2B: brain, liver, skeletal, smooth muscle, urinary bladder
KCNK1	GDB: 6045446	1q42-43	DPK, TWIK1	Kidney
KCNK2		1q41	TREK1	Brain
KCNK3	GDB: 9773281	2p23	TASK	Kidney

Therapeutic Target	Enzyme Family	Assay
Alzheimer's	CMGC	ERK2 (P42mapk)
Alzheimer's	Phospholipase	PLA2
Alzheimer's	Cyclooxygenases	COX2
Alzheimer's	CaMK	MARKI
Alzheimer's	CaMK	MARK2
Alzheimer's	AGC	PKCalpha
Alzheimer's	AGC	PKCgamma
Alzheimer's	AGC	PKCgamma
Alzheimer's	Cysteine proteases	caspase-3
Alzheimer's	Cysteine proteases	caspase-6
Alzheimer's	Aspartic proteases	BACE-1 (beta-secretase)
Alzheimer's	Aspartic proteases	cathepsin D
Alzheimer's	Aspartic proteases	cathepsin E
Alzheimer's	Metalloproteases	ACE
Alzheimer's	Metalloproteases	ACE
Alzheimer's	Metalloproteases	TACE
Alzheimer's	NO synthases	constitutive NOS (cerebellar)
Alzheimer's	Monoamine & neurotransmitter synthesis & metabolism	acetylcholinesterase
Alzheimer's	Monoamine & neurotransmitter synthesis & metabolism	COMT (catechol-O-methyl transferase)
Alzheimer's	Monoamine & neurotransmitter synthesis & metabolism	MAO-A
Alzheimer's	Monoamine & neurotransmitter synthesis & metabolism	MAO-B
Alzheimer's	Monoamine & neurotransmitter synthesis & metabolism	tyrosine hydroxylase
Alzheimer's	Phospholipase C	PLC
Alzheimer's	Miscellaneous enzymes	xanthine oxidase/ superoxide 02 - scavenging
Dependence/Addiction	AGC	PKA
Dependence/Addiction	AGC	PKCalpha
Dependence/Addiction	AGC	PKCbeta 1
Dependence/Addiction	AGC	PKCbeta 2
Dependence/Addiction	AGC	PKCdelta
Dependence/Addiction	Monoamine & neurotransmitter synthesis & metabolism	GABA transaminase
Dependence/Addiction	Cyclases	adenylyl cyclase (stimulated)
Dependence/Addiction	Phospholipase C	PLC
Dependence/Addiction	ATPase	ATPase (Na^+/K^+)
Inflammation/Arthritis/Allergy	RTK	EGFR kinase
Inflammation/Arthritis/Allergy	RTK	FLT-1 kinase (VEGFR1)
Inflammation/Arthritis/Allergy	RTK	KDR kinase (VEGFR2)
Inflammation/Arthritis/Allergy	CTK	Fyn kinase
Inflammation/Arthritis/Allergy	CTK	HCK
Inflammation/Arthritis/Allergy	CTK	Lek kinase
Inflammation/Arthritis/Allergy	CTK	Lyn kinase
Inflammation/Arthritis/Allergy	CTK	ZAP70 kinase
Inflammation/Arthritis/Allergy	CMGC	ERK2 (P42mapk)
Inflammation/Arthritis/Allergy	CMGC	JNK 1
Inflammation/Arthritis/Allergy	CMGC	JNK 2
Inflammation/Arthritis/Allergy	CMGC	P38alpha kinase
Inflammation/Arthritis/Allergy	Phospholipase	PLA2
Inflammation/Arthritis/Allergy	Cyclooxygenases	COX1
Inflammation/Arthritis/Allergy	Cyclooxygenases	COX2
Inflammation/Arthritis/Allergy	TXA2 synthetase	TXA2 synthetase
Inflammation/Arthritis/Allergy	CaMK	MAPKAPK2
Inflammation/Arthritis/Allergy	AGC	PKA
Inflammation/Arthritis/Allergy	Lipoxygenases	12-lipoxygenase
Inflammation/Arthritis/Allergy	Lipoxygenases	15-lipoxygenase
Inflammation/Arthritis/Allergy	Serine proteases	elastase
Inflammation/Arthritis/Allergy	Serine proteases	cathepsin G
Inflammation/Arthritis/Allergy	Serine proteases	kallikrein
Inflammation/Arthritis/Allergy	Serine proteases	tryptase
Inflammation/Arthritis/Allergy	Cysteine proteases	caspase-1
Inflammation/Arthritis/Allergy	Cysteine proteases	caspase-4
Inflammation/Arthritis/Allergy	Cysteine proteases	caspase-5
Inflammation/Arthritis/Allergy	Cysteine proteases	cathepsin B
Inflammation/Arthritis/Allergy	Cysteine proteases	cathepsin X
Inflammation/Arthritis/Allergy	Aspartic proteases	cathepsin E
Inflammation/Arthritis/Allergy	Metalloproteases	MMP-1
Inflammation/Arthritis/Allergy	Metalloproteases	MMP-2

-continued

Therapeutic Target	Enzyme Family	Assay
Inflammation/Arthritis/Allergy	Metalloproteases	MMP-3
Inflammation/Arthritis/Allergy	Metalloproteases	MMP-7
Inflammation/Arthritis/Allergy	Metalloproteases	MMP-8
Inflammation/Arthritis/Allergy	Metalloproteases	MMP-9
Inflammation/Arthritis/Allergy	Metalloproteases	MMP-13
Inflammation/Arthritis/Allergy	Metalloproteases	MT1-MMP (MMP-14)
Inflammation/Arthritis/Allergy	Metalloproteases	TACE
Inflammation/Arthritis/Allergy	Phosphatases	phosphatase CD45
Inflammation/Arthritis/Allergy	Phosphodiesterases	PDE2
Inflammation/Arthritis/Allergy	Phosphodiesterases	PDE4
Inflammation/Arthritis/Allergy	Phosphodiesterases	acid sphingomyelinase
Inflammation/Arthritis/Allergy	Monoamine & neurotransmitter synthesis & metabolism	HNMT (histamine N-methyltransferase)
Inflammation/Arthritis/Allergy	Miscellaneous enzymes	myeloperoxidase
Inflammation/Arthritis/Allergy	Miscellaneous enzymes	xanthine oxidase/ superoxide 02 - scavenging
Neuroprotection	RTK	TRKB
Neuroprotection	CMGC	CDK5
Neuroprotection	CMGC	DYRKla
Neuroprotection	CMGC	ERK1
Neuroprotection	CMGC	ERK2 (P42mapk)
Neuroprotection	MCGC	JCK 3
Inflammation/Arthritis/Allergy	Metalloproteases	MMP-13
Inflammation/Arthritis/Allergy	Metalloproteases	MT1-MMP (MMP-14)
Inflammation/Arthritis/Allergy	Metalloproteases	TACE
Inflammation/Arthritis/Allergy	Phosphatases	phosphatase CD45
Inflammation/Arthritis/Allergy	Phosphodiesterases	PDE2
Inflammation/Arthritis/Allergy	Phosphodiesterases	PDE4
Inflammation/Arthritis/Allergy	Phosphodiesterases	acid sphingomyelinase
Inflammation/Arthritis/Allergy	Monoamine & neurotransmitter synthesis & metabolism	HNMT (histamine N-methyltransferase)
Inflammation/Arthritis/Allergy	Miscellaneous enzymes	myeloperoxidase
Inflammation/Arthritis/Allergy	Miscellaneous enzymes	xanthine oxidase/ superoxide 02 - scavenging
Neuroprotection	RTK	TRKB
Neuroprotection	CMGC	CDK5
Neuroprotection	CMGC	DYRKla
Neuroprotection	CMGC	ERK1
Neuroprotection	CMGC	ERK2 (P42mapk)
Neuroprotection	MCGC	JCK 3
Neuroprotection	Cyclooxygenases	COXI
Neuroprotection	Cyclooxygenases	COX2
Neuroprotection	CaMK	CaMK2alpha
Neuroprotection	AGC	PKA
Neuroprotection	Cysteine proteases	caspase-3
Neuroprotection	Phosphodiesterases	PDEI
Neuroprotection	Phosphodiesterases	PDE6
Neuroprotection	NO synthases	constitutive NOS (endothelial)
Neuroprotection	NO synthases	constitutive NOS (cerebellar)
Neuroprotection	Monoamine & neurotransmitter syntheses & metabolism	acetylcholinesterase
Neuroprotection	Monoamine & neurotransmitter syntheses & metabolism	COMT (catechol-O-methyl transferase)
Neuroprotection	Monoamine & neurotransmitter syntheses & metabolism	GABA transaminase
Neuroprotection	Monoamine & neurotransmitter syntheses & metabolism	HNMT (histamine N-methyltransferase)
Neuroprotection	Monoamine & neurotransmitter syntheses & metabolism	MAO-A
Neuroprotection	Monoamine & neurotransmitter syntheses & metabolism	MAO-A
Neuroprotection	Monoamine & neurotransmitter syntheses & metabolism	PNMT (phenylethanoiamine-N-methyl transferase)
Neuroprotection	Monoamine & neurotransmitter syntheses & metabolism	tyrosine hydroxylase
Neuroprotection	Cyclases	guanylyl cyclase (basal)
Neuroprotection	Cyclases	guanylyl cyclase (stimulated)
Neuroprotection	ATPase	ATPase (Na+/K+)

-continued

Therapeutic Target	Enzyme Family	Assay
Neuroprotection	Miscellaneous enzymes	xanthine oxidase/superoxide 02 - scavenging
Parkinson	CMGC	JNK 1
Parkinson	Phospholipase	PLA2
Parkinson	Cyclooxygenases	COX2
Parkinson	Cysteine proteases	caspase-3
Parkinson	NO synthases	constitutive NOS (cerebellar)
Parkinson	Monoamine & neurotransmitter syntheses & metabolism	acetylcholinesterase
Parkinson	Monoamine & neurotransmitter syntheses & metabolism	COMT (catechol-O-methyl transferase
Parkinson	Monoamine & neurotransmitter syntheses & metabolism	MAO-A
Parkinson	Monoamine & neurotransmitter syntheses & metabolism	MAO-B
Cancer	RTK	Axl kinase
Cancer	RTK	c-kit kinase
Cancer	RTK	c-kit kinase
Cancer	RTK	EGFR kinase
Cancer	RTK	EphA1 kinase
Cancer	RTK	EphA3 kinase
Cancer	RTK	EphA4 kinase
Cancer	RTK	EphB2 kinase
Cancer	RTK	FGFR1 kinase
Cancer	RTK	FGFR2 kinase
Cancer	RTK	FGFR3 kinase
Cancer	RTK	FGFR4 kinase
Cancer	RTK	FLT-1 kinase (VEGFR1)
Cancer	RTK	FLT-3 kinase
Cancer	RTK	FLT-4 kinase (VEGFR3)
Cancer	RTK	Fms/CSFR kinase
Cancer	RTK	HER2/ErbB2 kinase
Cancer	RTK	HER4/ErbB4 kinase
Cancer	RTK	KDR kinase (VEGFR2)
Cancer	RTK	PDGFRalpha kinase
Cancer	RTK	PDGFRbeta kinase
Cancer	RTK	Ret kinase
Cancer	RTK	TIE2 kinase
Cancer	RTK	TRKA
Cancer	CTK	Abl kinase
Cancer	CTK	BLK
Cancer	CTK	BMX (Bk) kinase
Cancer	CTK	BRK
Cancer	CTK	BTK
Cancer	CTK	CSK
Cancer	CTK	FAK
Cancer	CTK	Fes kinase
Cancer	CTK	Fyn kinase
Cancer	CTK	JAK2
Cancer	CTK	JAK3
Cancer	CTK	Lck kinase
Cancer	CTK	PYK2
Cancer	CTK	Src kinase
Cancer	CTK	Syk
Cancer	CTK	Yes kinase
Cancer	CMGC	CDC2/CDK1 (cycB)
Cancer	CMGC	CDK2 (cycE)
Cancer	CMGC	CDK4 (cycD1)
Cancer	CMGC	CDK5
Cancer	CMGC	CK2 (casein kinase 2)
Cancer	CMGC	DYRKla
Cancer	CMGC	ERK1
Cancer	CMGC	ERK2 (P42mapk)
Cancer	CMGC	HIPK2
Cancer	CMGC	IKKalpha
Cancer	CMGC	IKKbeta
Cancer	CMGC	JNK 1
Cancer	CMGC	JNK 2
Cancer	CMGC	NEK1
Cancer	CMGC	NEK2
Cancer	CMGC	NEK4
Cancer	CMGC	p38alpha kinase

-continued

Therapeutic Target	Enzyme Family	Assay
Cancer	CMGC	p38beta 2 kinase (SAPK2b2)
Cancer	CMGC	p38delta kinase
Cancer	CMGC	p38ganuna kinase
Cancer	Cyclooxygenases	COX2
Cancer	CaMK	CaMK1delta
Cancer	CaMK	CaMK
Cancer	CaMK	CHK1
Cancer	CaMK	CHK2
Cancer	CaMK	DAPK1
Cancer	CaMK	DAPK2
Cancer	CaMK	MAPKAPK2
Cancer	CaMK	MAPKAPK3
Cancer	CaMK	MAPKAPK5 (PRAKO
Cancer	CaMK	MAARK1
Cancer	CaMK	MARK2
Cancer	CaMK	MARK4
Cancer	CaMK	Pim 1 kinase
Cancer	CaMK	Pirn2 kinase
Cancer	AGC	Akt1/PKBalpha
Cancer	AGC	Akt2/PKBbeta
Cancer	AGC	Akt3/PKBgamma
Cancer	AGC	AurA/Aur2 kinase
Cancer	AGC	AurB/Aur1 kinase
Cancer	AGC	AurC/Aur3 kinase
Cancer	AGC	P70S6Ke
Cancer	AGC	PDK1
Cancer	AGC	PKA
Cancer	AGC	PKCalpha
Cancer	AGC	PKCbeta 1
Cancer	AGC	PKCbeta 2
Cancer	AGC	PKCdelta
Cancer	AGC	PKCgamma
Cancer	AGC	PKG2
Cancer	AGC	ROCK1
Cancer	AGC	ROCK2
Cancer	AGC	RSK2
Cancer.	AGC	SGKI
Cancer	Lipoxygenases	12-lipoxygenase
Cancer	TKL	RAF-1 kinase
Cancer	STE	MEK1/MAP2KI
Cancer	STE	MKK4/JNK1
Cancer	STE	MKK6
Cancer	STE	PAK1
Cancer	STE	PAK2
Cancer	Serine proteases	elastase
Cancer	Serine proteases	cathepsin G
Cancer	Cysteine proteases	caspase-2
Cancer	Cysteine proteases	caspase-3
Cancer	Cysteine proteases	caspase-8
Cancer	Cysteine proteases	caspase-9
Cancer	Cysteine proteases	cathepin B
Cancer	Cysteine proteases	cathepsin H
Cancer	Cysteine proteases	cathepsin L
Cancer	Cysteine proteases	cathepsin X
Cancer	Aspartic proteases	cathepsin D
Cancer	Aspartic proteases	cathepsin E
Cancer	Metalloproteases	MMP-1
Cancer	Metalloproteases	MMP-2
Cancer	Metalloproteases	MMP-3
Cancer	Metalloproteases	MMP-7
Cancer	Metalloproteases	MMP-8
Cancer	Metalloproteases	MMP-9
Cancer	Metalloproteases	MMP-12
Cancer	Metalloproteases	MMP-13
Cancer	Metalloproteases	MT1-MMP (MMP-14)
Cancer	Metalloproteases	TACE
Cancer	Metalloproteases	MMP-1
Cancer	Phosphatases	phosphatase 1B
Cancer	Phosphatases	phosphatase 2B
Cancer	Phosphodiesterases	PDE2
Cancer	Phosphodiesterases	PDE4
Cancer	Phosphodiesterases	PDES
Cancer	Phosphodiesterases	acid spingomyelinase
Cancer	NO synthases	constitutive NOS (endothelial)
Cancer	NO synthases	constitutive NOS (cerebellar)

-continued

Therapeutic Target	Enzyme Family	Assay
Cancer	Cyclases	adenylyl cyclase (basal)
Cancer	Cyclases	adenylyl cyclase (stimulated)
Cancer	Phospholipase C	PLC
Cancer	Miscellaneous enzymes	myeloperoxidase
Cancer	Miscellaneous enzymes	xanthine oxidase/superoxide 02-scavenging
Diabetes	RTK	Ax1 kinase
Diabetes	RTK	EGFR kinase
Diabetes	RTK	IGFIR kinase
Diabetes	CMGC	ERK2 (P42mapk)
Diabetes	CMGC	Jnk1
Diabetes	Cyclooxygenases	COX2
Diabetes	TXA2 synthetase	TXA2 synthetase
Diabetes	CaMK	AMPKalpha
Diabetes	AGC	Akt1/PKBalpha
Diabetes	AGC	Akt2/PKBbeta
Diabetes	AGC	Akt3/PKBgamma
Diabetes	AGC	PDK1
Diabetes	AGC	PKA
Diabetes	AGC	PKCalpha
Diabetes	AGC	PKCbeta I
Diabetes	AGC	PKCbeta 2
Diabetes	AGC	PKCgamma
Diabetes	AGC	SGK2
Diabetes	Metalloproteases	ACE
Diabetes	Metalloproteases	MMP-1
Diabetes	Metalloproteases	MMP-2
Diabetes	Metalloproteases	MMP-3
Diabetes	Metalloproteases	MMP-7
Diabetes	Metalloproteases	MMP-8
Diabetes	Metalloproteases	MMP-9
Diabetes	Metalloproteases	MT1-MMP (MMP-14)
Diabetes	Metalloproteases	TACE
Diabetes	Phosphodiesterases	PDE3
Diabetes	Phosphodiesterases	PDE4
Diabetes	Phosphodiesterases	PDE5
Diabetes	NO synthases	constitutive NOS (endothelial)
Diabetes	Monoamine & neurotransmitter synthesis & metabolism	acetylcholinesterase
Diabetes	Monoamine & neurotransmitter synthesis & metabolism	GABA transaminase
Diabetes	Monoamine & neurotransmitter synthesis & metabolism	MAO-B
Diabetes	Cyclases	adenylyl cyclase (basal)
Diabetes	Miscellaneous enzymes	acetylCoA synthetase
Diabetes	Miscellaneous enzymes	HMG-CoA reductase
Diabetes	Miscellaneous enzymes	xanthine oxidase/superoxide 02-scavenging
Metabolic Diseases	Cyclooxygenases	COX2
Metabolic Diseases	AGC	PICA
Metabolic Diseases	Metalloproteases	ACE
Metabolic Diseases	Phosphodiesterases	PDE3
Metabolic Diseases	Phosphodiesterases	PDE4
Metabolic Diseases	NO synthases	constitutive NOS (endothelial)
Metabolic Diseases	Miscellaneous enzymes	acetylCoA synthetase
Metabolic Diseases	Miscellaneous enzymes	HMG-CoA reductase
Metabolic Diseases	Miscellaneous enzymes	xanthine oxidase/superoxide 02-scavenging
Obesity	CTK	PYK2
Obesity	CMGC	JNK1
Obesity	CaMK	AMPJakoga
Obesity	AGC	PKA
Obesity	Metalloproteases	ACE
Obesity	Metalloproteases	ACE
Obesity	Phosphatases	phosphatase IB
Obesity	Phosphodiesterases	PDE2
Obesity	Phosphodiesterases	PDE3
Obesity	Monoamine & neurotransmitter synthesis & metabolism	acetylcholinesterase
Obesity	ATPase	ATPase (Na+/K+)

-continued

Therapeutic Target	Enzyme Family	Assay
Obesity	Miscellaneous enzymes	HMG-CoA reductase
Reproduction	Phospholipase	PLA2
Reproduction	Cyclooxygenases	COX1
Reproduction	Cyclooxygenases	COX2
Reproduction	Phosphodiesterases	PDE5
Reproduction	NO synthases	constitutive NOS (endothelial)
Reproduction	Cyclases	guanylyl cyclase (stimulated)
Cystic Fibrosis	Phospholipase	PLA2
Cystic Fibrosis	TXA2 synthetase	TXA2 synthetase
Cystic Fibrosis	AGC	PKA
Cystic Fibrosis	AGC	PKCbeta 1
Cystic Fibrosis	AGC	PKCbeta 2
Cystic Fibrosis	Serine proteases	elastase
Cystic Fibrosis	Serine proteases	cathepsin G
Cystic Fibrosis	Metalloproteases	MMP-2
Cystic Fibrosis	Phosphodiesterases	PDE3
Cystic Fibrosis	Phosphodiesterases	PDE5
Cystic Fibrosis	Cyclases	adenylyl cyclase (stimulated)
Cystic Fibrosis	Phospholipase C	PLC
Cystic Fibrosis	Miscellaneous enzymes	myeloperoxidase
Immunosuppression Profile	RTK	EGFR kinase
Immunosuppression Profile	CTK	JAK3
Immunosuppression Profile	CMGC	ERK2 (P42mapk)
Immunosuppression Profile	Cyclooxygenases	COX1
Immunosuppression Profile	Cyclooxygenases	COX2
Immunosuppression Profile	Serine proteases	elastase
Immunosuppression Profile	Serine proteases	cathepsin G
Immunosuppression Profile	Serine proteases	tryptase
Immunosuppression Profile	Cysteine proteases	cathepsin B
Immunosuppression Profile	Metalloproteases	ECE-1
Immunosuppression Profile	Metalloproteases	ECE-1
Immunosuppression Profile	Metalloproteases	MMP-1
Immunosuppression Profile	Metalloproteases	MMP-2
Immunosuppression Profile	Metalloproteases	MMP-9
Immunosuppression Profile	Phosphatases	phosphatase CD45
Immunosuppression Profile	Phosphodiesterases	PDE4
Immunosuppression Profile	Phosphodiesterases	acid spingomyelinase
Immunosuppression Profile	Cyclases	adenylyl cyclase (basal)
Immunosuppression Profile	Cyclases	adenylyl cyclase (stimulated)
Migraine	Cyclooxygenases	COX2
Migraine	NO synthases	constitutive NOS (cerebellar)
Migraine	Monoamine & neurotransmitter synthesis & metabolism	GABA transaminase
Migraine	Cyclases	guanylyl cyclase (stimulated)
Pain	CMGC	ERK2 (42mapk)
Pain	Phospholipase	PLA2
Pain	Cyclooxygenases	COXI
Pain	Cyclooxygenases	COX2
Pain	AGC	PICA
Pain	Serine proteases	elastase
Pain	Metalloproteases	MMP-1
Pain	Metalloproteases	MMP-2
Immunosuppression Profile	Serine proteases	elastase
Immunosuppression Profile	Serine proteases	cathepsin G
Immunosuppression Profile	Serine proteases	tryptase
Immunosuppression Profile	Cysteine proteases	cathepsin B
Immunosuppression Profile	Metalloproteases	ECE-1
Immunosuppression Profile	Metalloproteases	ECE-1
Immunosuppression Profile	Metalloproteases	MMP-1
Immunosuppression Profile	Metalloproteases	MMP-2
Immunosuppression Profile	Metalloproteases	MMP-9
Immunosuppression Profile	Phosphatases	Phosphatase CD45
Immunosuppression Profile	Phosphodiesterases	PDE4
Immunosuppression Profile	Phosphodiesterases	acid spingomyelinase
Immunosuppression Profile	Cyclases	adenylyl cyclase (basal)
Immunosuppression Profile	Cyclases	adenylyl cyclase (stimulated)
Migraine	Cyclooxygenases	COX2
Migraine	NO synthases	constitutive NOS (cerebellar)

-continued

Therapeutic Target	Enzyme Family	Assay
Migraine	Monoamine & neurotransmitter synthesis & metabolism	GABA transaminase
Migraine	Cyclases	guanylyl cyclase (stimulated)
Pain	CMGC	ERK2 (42mapk)
Pain	Phospholipase	PLA2
Pain	Cyclooxygenases	COXI
Pain	Cyclooxygenases	COX2
Pain	AGC	PICA
Pain	Serine proteases	elastase
Pain	Metalloproteases	MMP-1
Pain	Metalloproteases	MMP-2
Pain	Metalloproteases	MMP-3
Pain	Metalloproteases	MMP-7
Pain	Phosphodiesterases	PDE4
Pain	NO synthases	constitutive NOS (endothelial)
Pain	NO synthases	constitutive NOS (cerebellar)
Pain	Monoamine & neurotransmitter synthesis & metabolism	GABA transaminase
Pain	Monoamine & neurotransmitter synthesis & metabolism	MAO-A
Pain	Monoamine & neurotransmitter synthesis & metabolism	MAO-B
Pain	Monoamine & neurotransmitter synthesis & metabolism	tyrosine hydroxylase
Pain	Miscellaneous enzymes	xanthine oxidase/superoxide 02 - scavenging

The invention now being generally described, it will be more readily understood by reference to the following examples which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the invention in any way.

EXAMPLES

Ion-Selective Polymer Solution. The ion-selective polymer solution was made from the following components: 30 mg High Molecular Weight Polyvinyl Chloride, 60 mg Bis-2-Sebacate, 0.1 mg Sodium Ionophore X, 0.1 mg Sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate, and 0.1 mg Chromoionophore I. The combined reagents were stirred in 500 μL of tetrahydrofuran (THF) to afford a homogenous solution.

Ion-Selective Sensor Fabrication. Quantum dots (ITK organic 655, Invitrogen) were flocculated in a methanol/isopropanol mixture with the addition of toluene in a 1:1 (v:v) ratio of toluene:quantum dot solution. The supernatant was removed and the quantum dots were resuspended in THF containing 3.3 mM 1-decanethiol. To the quantum dot solution (0.2 nMoles) was added the ion-selective polymer solution (17.2 nMoles Chromoionophore I, 50 μl) and the mixture was stirred.

Immobilized Polymer Matrix of Sensors. To form an immobilized polymer matrix of sensors, 1 μl of the polymer/quantum dot mixture was dropped onto a 5 mm glass coverslip to afford a thin homogenous matrix. Individual coverslips could then be placed in 96-well plates and experiments carried out.

Particle Sensors. To form particle sensors, dichloromethane was added to the polymer/quantum dot mixture in a 1:1 (v:v) ratio. This solution was then added directly to 5 mL of H_2O containing 200 μg of dissolved 1,2-Diacyl-sn-Glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethyleneglycol)-550] (PEG-lipid) by injecting through a pipette tip during sonication. Sonication was performed with a probe-tip sonicator (Branson) at 15% amplitude during and after addition for a total of 3 minutes, resulting in plasticized polymer nanosphere formation. The solution was then passed through a 0.2 μm syringe filter (Acrodisc, Gelman Laboratory) to remove large particles.

Particle Sizing and Zeta Potential. Particle size and zeta potential of the sensors were determined using a nanosizer (Nano Series ZS90, Malvern).

Calibration and Selectivity. Selectivity was determined using immobilized polymer matrix sensors. Parallel measurements were taken during addition of increasing ion concentration in HEPES (10 mM) buffered solution (pH=7.4) for response to sodium and potassium (n=4 each). Response was determined by expressing the data as $\alpha=(I-I_{min})/(I_{max}-I_{min})$. I is the intensity at the given ion concentration, I_{min} is the intensity at the minimum signal (0 Na^+), and I_{max} is the intensity at the maximum signal (1 M Na^+). Data were acquired in a Spectramax Gemini EM microplate fluorometer (Molecular Devices) exciting at 405 nm and emitting at 655 nm. The immobilized polymer matrix sensors were pretreated with HEPES buffered solution at pH=5.0, the pH of the sensors in solution. The baseline was then established in HEPES buffered solution at pH=7.4. Sodium and potassium solutions in the range of 1 mM to 1 M were added and the sensor was allowed to equilibrate before measurements were made. Response was determined by fitting a sigmoidal curve to the plot of α vs. ion concentration using Origin software, FIG. **4**.

In order to prevent dilution effects, the polymer matrix with no biocompatible coating was immobilized to a glass coverslip for calibration and selectivity measurements. The dynamic range of the sensor was found to be 1 mM to 1 M, shown spectrally in FIG. **5**B. By adjusting the ratio of components, the concentration range was tuned to maximize the resolution at typical levels of intracellular sodium. In this case, the resolution was 80 μM at 17 mM, the center of the

dynamic range. This means that a change in fluorescence intensity of 1% would correspond to a change in concentration of 80 μM, as measured on a fluorescence plate reader.

Ratiometric Measurements Immobilized polymer matrix sensors containing both 545 nm and 655 nm quantum dots was made in a similar fashion to the method described above. In this case, 1 nmole of each colored (i.e., emission wavelength) quantum dot was used, giving a total of 2 nmoles of quantum dots just as above. The sensors were calibrated with sodium ions while recording emission at 545 and 655 nm in the fluorometer, using an excitation wavelength of 405 nm. The ratio of 655/545 was taken, averaged over the data set and plotted using the graphing software program Origin. A sigmoidal curve was fit to the data and a half-maximal response was determined (FIG. **5**B).

A control ion-selective polymer matrix was made similarly to polymer matrix described above, however the control polymer matrix did not contain Chromoionophore I. Immobilized polymer matrix sensors were made as described above. The response was determined by measuring fluorescence over 30 minutes after addition of 200 mM sodium solution. (This was performed in parallel with standard immobilized polymer matrix sensors to analyze the response time).

Spectral Overlap. Absorbance characteristics of the chromoionophore were obtained by creating sensors without quantum dots. The sensors were placed in a 96-well plate and absorbance was measured at wavelengths ranging from 500 nm to 700 nm. This was performed in the presence of 0, 1, 10, 100, and 1000 mM sodium solution in pH 7.4 Hepes buffer. From these spectral results, quantum dots were selected that emit fluorescence at a wavelength that coincides with the chromoionophore absorbance wavelengths. The overlap was confirmed by creating a sensor without chromoionophore but with quantum dots at the ratio mentioned above. A fluorescent spectrum was then measured by exciting at 400 nm and collecting emission from 600 nm to 700 nm in steps of 5 nm. In the absence of the chromoionophore, the quantum dot sensors fluoresced at an intensity that was not affected by the presence or absence of sodium ions.

A quantum dot with fluorescence maximum at 655 nm and a chromoionophore in a sensor that absorbs fluorescence at low sodium ion concentrations is depicted in FIG. **6**. The absorbance of the chromoionophore at 655 nm (gray lines, FIG. **6**) decreases as sodium concentration increases, resulting in an increase in fluorescent signal directly related to increasing sodium concentration. Note the preferred overlap of the quantum dot emission (red line, FIG. **6**).

Particle sensors without quantum dots: A polymer cocktail was formulated using, for example, traditional amounts of the sensor components (PVC, plasticizer, ionophore, chromoionophore and ionic additive) in a 1:1 (v:v) THF/dichloromethane solution. A probe-tip sonicator was used to sonicate an aliquot of 100 μL of polymer solution in 5 mL of buffer containing 0.1% PEG modified lipid. The nanosensors were dried by passing the particle solution through a nitrogen feed airbrush.

Surface Chemistry: The surface chemistry of the sensor can be changed by varying the concentration of the functionalized PEG incorporated onto the sensor. As above, zeta potential can be used to analyze the effectiveness of the coating at any given functionalized PEG concentration. The concentration of the functionalized PEG may be changed to optimize the properties of the sensors to their intended use.

Incorporation of Surface modifier onto Nanosensor Surface: The PEG lipid molecule PEG2000 PE {1,2-Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-2000] (Ammonium Salt)} (Avanti Polar Lipids, Alabaster Ala.) can be used to attach functional groups. The amine functional group is also available through Avanti Polar Lipids, DSPE-PEG(2000)Amine {1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-[Amino(Polyethylene Glycol)2000] (Ammonium Salt)}, and requires no alterations.

The functionalized PEG can be dissolved into HEPES Buffered Saline (HeBS) and added to a solution containing sensor. The mixture may be mixed, e.g., with a vortexer for 1-2 minutes, to ensure ample interaction time and destabilization of aggregates. The resulting sensor-PEG can be subjected to acidification (decrease of solution pH from 7.4-5.5) and mechanical stress (trituration). The zeta potential can be measured (zetasizer) before and/or after acidification and mechanical stress to determine the surface concentration of the SM. This may correlate to the ability of the functionalized PEG to coat the surface of the sensor and withstand chemical and mechanical changes associated with the endocytotic pathway.

Biocompatibility. Biocompatibility was determined by incubating the sensors with HEK 293 cells (ATCC). The cells were trypsonized from normal culture and pipetted into a clear 96-well plate at a concentration of 30,000 cells/well. The cells were grown overnight in 300 μL of media to allow attachment to the plate. An aqueous solution (20 μL) containing 10^{11} particle sensors/mL was added to each well. For control experiments 20 μL of distilled water was used. Different particles were used to compare to the particle sensors. Particles used for comparison include: gold nanoparticles (colloidal gold 100 nm, SPI) and FluoSpheres (20 nm carboxylate modified microspheres, Invitrogen). Each type of nanosensor was tested on 8 wells of cells. The nanosensors were incubated with the cells overnight and the media was changed the following day.

At 24, 48, and 72 hours following washing, an MTT assay (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was performed (In vitro toxicology assay kit, Sigma). The cells were incubated with MTT for 2 hours. The MTT reduction product formazan was then dissolved and the absorbance of each well was read at 570 and 690 nm. The 690 nm absorbance served as background and was subtracted from the 570 nm value. The data was then averaged and compared to control to generate FIG. **7**.

Loading sensors without quantum dots into cells: One method used for loading the cell involved incubating a solution of sensors in media with the cells overnight. The following day, the cells were washed 3× with PBS buffer. The cells were then imaged on the confocal microscope, with excitation 633 nm and emission 670-690 nm indicating that some of the polymer nanosensor had loaded into the cell, with plenty in the cytosol, but the image indicated a distribution in larger bundles clustered around the nucleus, FIG. **8**.

Targeted nanosensors The surface chemistry of a nanosensor can be modified as in this example to promote loading of the sensors into the cytosol of cells. A targeting moiety or peptide such as an amine, or melittin can be incorporated onto the surface of the sensor by attaching the functional group to the head of a polyethylene glycol (PEG) SM. The lipid portion of the SM can then be incorporated into the nanosensor by interaction with the polymer membrane. The lipid molecule may maintain similar properties regardless of the functional group attached to the PEG, therefore, this step can be the same for the various functional groups.

The nanosensor can be coated with the targeting moiety-bound SM by vortexing. This method should apply the least amount of stress to the nanosensor, while allowing for the necessary interaction of the two components to form a functionalized nanosensor. The amount of targeting moiety-

bound SM can be limiting in the interaction and therefore little residual labile targeting moiety-bound SM should remain in solution. Additionally, it is assumed that the nanosensor can be evenly coated and coating may be a function of the concentration of targeting moiety-bound SM. Once the incorporation of the targeting moiety-bound SM onto the surface of the nanosensor has been established, the surface chemistry of the nanosensor will be tested.

The targeting moiety-bound SM coated nanosensor can first be tested for response to the ion of choice outside of the cell. Testing the location of the nanosensor within the cell may be performed by, for example, LSCM with the addition of organelle specific dyes and TEM for co-localization studies. When it has been determined that the functionalized nanosensor are indeed in the cytosol, the ability to respond to increases and decreases in the concentration of the ion of interest can be addressed.

Loading sensors into cells: A method for loading sensors into cells involves injecting the sensors into the cytosol of the HEK 293 cells via a 2 MOhm resistance patch pipette. The sensor solution may be diluted, e.g., one to one with a 2× intracellular solution. Once a GOhm seal has been achieved, the membrane ruptures and the sensors diffuse into the cytosol of the cell. No pressure need be applied to the patch pipette solution; rather the sensors enter the cytoplasm by simple diffusion. Time course experiments can be performed by acquiring sensor signal every minute over the course of 30 minutes to evaluate the rate of diffusion out of the patch pipette and homogeneity of distribution once inside the cytosol. Patching of cells is discussed in detail below.

Ability to Load into Cell: The sensors can be loaded into the test cell, such as HEK 293 or HL-1 cells. HEK 293 cells are maintained by standard cell culture and HL-1 cells are maintained similarly with alterations. Cells may be plated, e.g., onto 25 mm glass cover slips in 6 well plates at 25% confluence the day before experiments are to be carried out. When using HEK 293 cells the cover slips may be coated with poly-L-lysine, while cover slips for HL-1 cell experiments may be coated with gelatin and fibronectin.

The sensors in a HeBS solution may be added to 2 mL of cell culture media (at volumes not exceeding a ratio of 1:10) to replace the media in each well. The sensors can be incubated with the cells at 37° C. 5% CO_2, e.g., 10 min-24 hrs. Following incubation, the cover slips may be washed with HeBS containing 10 mM Glucose warmed to 37° C. and transferred to a microscope chamber. The chamber may be filled with HeBS with glucose and placed onto the microscope (microscope experiments may be performed on the LSCM). Data may be acquired using LSCM. Images may be acquired using a plan-apochromatic 63×1.4 NA oil immersion lens. Excitation/Emission settings may be selected according to the type of sensors being tested, e.g., for particle sensors-PEG Ex/Em of 633/670-690 nm. Loading may be evaluated by determining the quantity of sensors in each cell. A lack of nuclear loading may indicate either intracellular loading or plasma membrane incorporation.

Ability to Locate Into cytosol: The sensors may be analyzed for their ability to release from endosomes and enter the cytosol. Both HEK 293 and HL-1 cells may be prepared for microscopy according to the methods above. Additionally, the sensors may be loaded as described above, optionally with organelle-specific dyes. LSCM images may be acquired using the same microscope configuration described above. Cells may be loaded with sensors as described above and fixed for analysis of cytosolic location by TEM.

Imaging of sensors: After loading via patch pipette the sensors for both simultaneous patch experiments and independent imaging experiments may be performed. Images can be acquired with a standard CCD camera (CoolSnap HQ, Roper Scientific) or a high speed camera (Cooke, for channel experiments) attached to a Zeiss Axiovert 200 microscope. A standard FITC cube (Chroma) may be used when imaging CoroNa Green. Channel activity may be controlled with the patch setup and fluorescence detection may be timed to coordinate with channel opening.

Intracellular calibration: Calibration experiments can be performed in HEK 293 cells. The SENSORs can be calibrated in the cytosol after injection loading with a pipette. The sodium ionophore gramicidin (10 μM, Sigma) can be used to transport sodium across the membrane and Strophanthidin (100 μM, Sigma) can be used to deactivate any Na—K ATPase in the cells. Two solutions can be made that contain 140 mM Sodium (30 mM NaCl and 110 mM sodium gluconate) and zero Sodium (30 mM KCl and 110 mM potassium gluconate, to maintain ionic balance). Both solutions may also contain 10 mM HEPES, 10 mM glucose and, 1 mM EGTA (sigma) and a pH of 7.4. They can be mixed to achieve the desired concentration of sodium and perfused into the microscope chamber. Acquisitions of data may be made every 5 seconds and the sodium concentration may only be switched after a plateau in signal has been achieved for over two minutes. Selectivity can be determined by performing a calibration response to potassium. In this case, however, valinomycin (Sigma) may be used instead of gramicidin, all other conditions being the same.

Response repeatability can be determined using the conditions in the sodium calibration. The extracellular concentration of sodium can be switched back and forth from zero sodium to 50 mM sodium every ten minutes over the course of an hour.

Intracellular Response to Ion of Interest: Experiments may be performed to determine responsiveness of the nanosensors in the cytosol. Cells prepared for microscope experiments and functionalized nanosensors may be loaded as described above. Nanosensors may be imaged on a LSCM as described above. A description of methods suitable to characterize sodium nanosensor response follows; when using other ion-specific nanosensor, different ionophores can be employed.

LSCM images may be from nanosensor-loaded cells in HeBS containing zero sodium. A sodium ionophore such as Valinomycin (10 μM) (Sigma) may be added to equilibrate the concentration of sodium between the extracellular solution and the cytosol. Sodium concentrations may be increased in a step-wise manner by addition of a high sodium (1 M) stock HeBS. Images may be acquired after the system is allowed to reach equilibrium (~2-3 min) and intensity of the nanosensors can be measured. The sodium concentration may be raised, e.g., to 1 M, to establish a maximum concentration value of intensity. The data may then be correlated to the both the minimum intensity and maximum intensity and a calibration curve can be generated.

In a similar fashion, selectivity of the cytosolic nanosensor may be determined using interfering ions and their corresponding ionophores. The addition of interfering ions and the ionophore may be added while performing the calibration mentioned above. The acquired calibration curve may then be compared to that generated from sodium ion alone and a selectivity coefficient can be determined.

Nanosensor-loaded cells may also be subjected to whole-cell patch-clamp. The cells can be exposed to a drug to induce sodium flux across plasma membrane channels. Channel activity using patch-clamp can be recorded simultaneously

with nanosensor fluorescence. This allows a direct comparison of this method to measure ion flux with the instant method discussed herein.

Whole Cell Patching: Recombinant HEK 293 cells expressing $Na_v1.7$ can be used to analyze sodium detection of intracellular nanosensors. Standard whole cell voltage clamp protocols may be employed to assess channel current density, voltage-dependent activation, inactivation, and the time course of recovery from inactivation. Ionic currents may be recorded with whole-cell voltage clamp methods, using an Axopatch-200B amplifier (Axon Instruments). Borosilicate glass electrodes with tip resistances 1-3 Mohm can be used. Command pulses may be generated by 12-bit digital-to-analog converter controlled by pCLAMP6 software (Axon Instruments). Experiments may be conducted at 36° C. To measure activity of the voltage-gated sodium channels, currents can be recorded following a step change of the holding potential from −120 mV to −20 mV test potential. The external solution may contain (mmol/L): 30 NaCl, 110 CsCl, 1.8 $CaCl_2$, 2 $CdCl_2$, 1 $MgCl_2$, 10 HEPES, 10 glucose, 1 4-AP. Intracellular solution may contain (mmol/L): 10 NaCl, 130 CsCl, 5 EGTA, 10 HEPES, 10 glucose. Inactivation and activation kinetics can be analyzed. Nanosensors may be introduced via patch pipette in the whole cell configuration, and maximum amplitude of the sodium current can be measured simultaneously with measurement of sodium flux optical recording from the nanosensors.

The same experiments may be repeated with recombinant $K_v1.3$ HEK 293 cells to demonstrate specificity of the sodium-sensitive nanosensors. In this case the cells may be hyperpolarized to below the reversal potential for potassium to allow for potassium influx into the cell. To measure the potassium currents, the extracellular solution may contain (mmol/L) NaCl 136, KCl 5.4, $MgCl_2$ 1, $CaCl_2$ 1, NaH_2PO_4 0.33, HEPES 5, and dextrose 10 (pH 7.35, NaOH). For delayed rectifier current recording, nifedipine (5 μmol/L), 4-aminopyridine (2 mmol/L), and atropine (200 nmol/L) can be added to suppress L-type calcium current ($I_{Ca,L}$), transient outward current (I_{to}), and 4-aminopyridine-dependent muscarinic K^+ currents. Dofetilide (1 μmol/L) can be added for I_{Ks} recording. For I_{to} recording, nifedipine may be replaced by $CdCl_2$ (200 μmol/L). Na^+ current (I_{Na}) contamination may be avoided by substitution of equimolar Tris-HCl for NaCl and use of tetrodotoxin. A suitable internal solution for K^+-current recording may contain (mmol/L) K-aspartate 110, KCl 20, $MgCl_2$ 1, MgATP 5, LiGTP 0.1, HEPES 10, Na-phosphocreatinine 5, and EGTA 5.0 (pH 7.3, KOH).

Validation of sensor response with molecular dyes: The same experiments carried out on sensors may be performed using a fluorescein detection microscope cube. The cell impermeant CoroNa Green salt can be loaded through the patch pipette to determine if patch pipette loading alters the location and response of the dye.

Characterization of sensor function: Blockade of sodium channel using lidocaine and STX can reduce sodium flux into the cell, resulting in decrease in signal amplitude using optical recordings from the sensors. Recombinant $Na_v1.7$ HEK 293 cells may be subject to whole cell patch clamp, and the sensors may be introduced via the patch clamp. Lidocaine or STX may be perfused into the patch chamber and peak amplitude of the sodium current can be determined both by patch clamp (using standard voltage-dependent activation protocol) and optical recording (sensors) before and after infusion of the agent.

Live/Dead Assays After Nanosensor Loading: A fluorescence live/dead assay, consisting of calcein to stain living cells and ethidium bromide to stain the nuclei of dead cells, was performed in order to determine the viability of cells loaded with nanosensors, FIG. **9**. The staining procedure included incubation of nanosensor-loaded cells (overnight loading, as above) in 8 μM of calcein and 8 μM of ethidium bromide for 20 minutes at 37° C. 5% CO_2, then rinsing 3×. The cells were then imaged on the confocal microscope. In FIG. **9** the green indicates healthy cells, while the red stains the nuclei of dead cells. No difference in the ratio of live to dead cells was noted between nanosensor loaded cells and control (no nanosensors). Following an implementation of this method, it was noted that the number of living cells was not different from control cells (not shown) which contained no nanosensors.

Nanosensors in Cardiac Cells: Isolated neonatal rat ventricular myocytes were plated onto laminin coated 25 mm coverslips. Electroporation was performed in a custom chamber with custom parallel electrodes spaced at 1 cm. The cells were porated in a Ringer's solution containing 1:10 volume ratio of nanosensor solution. 800 V pulses were applied for 20 μseconds 8 times using an electroporator (Harvard Apparatus). The cells were then allowed to recover for 10 minutes before imaging. Images were taken on a confocal microscope (LSM 510 Meta, Zeiss) exciting at 488 nm and emitting at 650-690, 63× oil immersion objective. As can be seen in FIG. **10**A, the nanosensors loaded into the cardiac cells efficiently using electroporation.

As the cells "beat" during electrical stimulation in cardiac cells, the fluorescence from the sodium sensors was collected and was seen to oscillate at 1 Hz, the pacing frequency. In FIG. **10**B, the fluorescence collected from a nanosensor during stimulation, is charted. The changes of fluorescence observed are attributed to oscillation of sodium, as the changes occur at the pacing frequency of the cells. The data is the average of three time segments of the same experiment (data from time 0-3 seconds was averaged with data 3-6 seconds and data 6-9 seconds), and base-line corrected to account for photobleaching.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

The invention claimed is:

1. A sensor comprising:

a) a fluorescence source;

b) a polymer, an ionophore and a chromoionophore, wherein in an initial state photons emitted from the fluorescence source are absorbed by the chromoionophore, and when the ionophore associates with an ionic analyte, the chromoionophore stops absorbing photons emitted by the fluorescence source; and

c) an internalizing moiety that localizes the sensor within the cytosol of a cell.

2. A sensor comprising:

a) a fluorescence source;

b) a polymer, an ionophore and a chromoionophore, wherein in an initial state photons emitted from the fluorescence source are not absorbed by the chromoionophore, and when the ionophore associates with an ionic analyte, the chromoionophore absorbs photons emitted by the fluorescence source; and

c) an internalizing moiety that localizes the sensor within the cytosol of a cell.

3. An apparatus for measuring a characteristic within a living cell, the apparatus comprising:

(a) a sensor of claim **1** or **2**; and

(b) circuitry for detecting the photons.

4. The sensor of claim 1 or 2, wherein the ionic analyte is K^+, Na^+, Ba^{2+}, Li^+, NH_4^+, or Ca^{2+}.

5. The sensor of claim 1 or 2, wherein the ionic analyte is Cl^- or NO_3^-.

6. The sensor of claim 1, wherein the fluorescence source comprises one or more quantum dots.

7. The sensor of claim 1, wherein the fluorescence source comprises one or more fluorescent dyes.

8. The sensor of claim 2, wherein the fluorescence source comprises one or more quantum dots.

9. The sensor of claim 2, wherein the fluorescence source comprises one or more fluorescent dyes.

10. The sensor of claim 1 or 2, wherein the sensor comprises multiple quantum dots, chromoionophore and ionophore.

11. The sensor of claim 1 or 2, wherein the sensor is a film.

12. The sensor of claim 1 or 2, wherein the sensor is a nanoparticle.

13. The sensor of claim 12, wherein the diameter of the nanoparticle is between 5 nm and 300 nm.

14. The sensor of claim 12, wherein the diameter of the nanoparticle is between 20 nm and 200 nm.

15. The sensors of claim 12, wherein the nanosensor comprises only one quantum dot and has a diameter between 5 nm and 50 nm.

16. The sensor of claim 1 or 2, wherein the sensor further comprises a targeting moiety.

17. The sensor of claim 16, wherein the targeting moiety is bound to any of the polymer matrix, the surface modifier, the internalizing moiety or a combination thereof.

18. The sensor of claim 16, wherein the targeting moiety is selected from small molecules, proteins, sugars or any combination thereof.

19. The sensor of claim 18, wherein multiple targeting moieties are bound to one or more components of the sensor.

20. The sensor of claim 1 or 2, wherein the internalizing moiety is bound to any of the polymer matrix, the surface modifier, the targeting moiety or a combination thereof.

21. The sensor of claim 1 or 2, wherein the internalizing moiety is selected from an amine, antepennepedia, mastoparan, melittin, bombolittin, delta hemolysin, pardaxin, *Pseudomonas* exotoxin A, clathrin, Diphtheria toxin, C9 complement protein, or a fragment of one of the preceding proteins, or a combination thereof.

22. The sensor of claim 21, wherein multiple internalizing moieties are bound to one or more components of the sensor.

23. The sensor of claim 1 or 2, wherein the internalizing moiety also functions as a targeting moiety.

24. The sensor of any of claims 1-5, further comprising a surface modifier.

25. The sensor of claim 24, wherein the surface modifier comprises a hydrophilic portion and a hydrophobic portion.

26. The sensor of claim 25, wherein the hydrophilic portion of the surface modifier is polyethylene glycol.

27. The sensor of claim 25, wherein the hydrophobic portion of the surface modifier is a lipid.

28. The sensor of claim 25, wherein the hydrophobic portion of the surface modifier and the hydrophilic portion of the surface modifier are bound together through a linker.

29. The sensor of claim 28, wherein the linker is a covalent bond, a phosphate or a ceramide.

30. A method for detecting a characteristic of a living cell, the method comprising:

(a) contacting the cell with at least one sensor, wherein the sensor comprises a polymer, a fluorescence source that fluoresces at a first wavelength, an internalizing moiety that localizes the sensor within the cytosol of a cell and a chromoionophore that absorbs photons of the first wavelength in one state and does not absorb photons of the first wavelength in a second state, wherein the states are indicative of the characteristic of the cell, and

(b) exciting the fluorescence source with a light source causing the fluorescence source to fluoresce, and

(c) detecting a signal from the sensor.

31. The method of claim 30, wherein the characteristic is the presence of an ionic analyte.

32. The method of claim 31, wherein the sensor further comprises an ionophore that selectively binds the ionic analyte.

33. The method of claim 32, wherein in one state the chromoionophore is deprotonated and in the second state the chromoionophore is protonated.

34. The method of claim 32, wherein the ionic analyte is K^+, Na^+, Ba^{2+}, Li^+, NH_4^+, or Ca^{2+}.

35. The method of claim 32, wherein the ionic analyte is Cl^- or NO_3^-.

36. The method of claim 33, wherein the ionic analyte is cationic and when ionic analyte is associated with the ionophore, the chromoionophore is deprotonated, and when the ionic analyte is not associated with the ionophore, the chromoionophore is protonated.

37. The method of claim 33, wherein the ionic analyte is anionic and when the ionic analyte is associated with the ionophore, the chromoionophore is protonated, and when the ionic analyte is not associated with the ionophore, the chromoionophore is deprotonated.

38. The method of claim 30, further comprising stimulating the cell so as to affect the characteristic.

39. The method of claim 38, wherein stimulating includes contacting the cell with at least one of a compound, a pathogen, a pharmaceutical compound, or a potential toxin.

40. The method of claim 30, wherein contacting the cell comprises placing at least one sensor in proximity to the cell.

41. The method of claim 30, wherein contacting the cell comprises coupling the at least one sensor to the exterior membrane of the cell.

42. The method of claim 41, wherein contacting the cell comprises coupling the at least one sensor to the exterior membrane of the cell proximate to an ion channel of the cell.

43. The method of claim 42, wherein the at least one sensor is coupled to the external membrane of the cell via an antibody that specifically binds the ion channel.

44. The method of claim 30, wherein contacting the cell comprises introducing the sensor into the cell.

45. The method of claim 44, wherein introducing the sensor into the cell comprises incubating the cell with the sensor in a medium.

46. The method of claim 30, wherein the characteristic is the presence of a non-ionic product, and wherein the method further comprises ionizing said non-ionic product.

47. The method of claim 30, wherein the cell is in an animal.

48. The method of claim 30, wherein the fluorescence has an intensity indicative of the concentration of ionic analyte.

49. The method of claim 30, wherein the signal comprises the intensity of the fluorescence of the at least one sensor.

50. The method of claim 49, wherein detecting the signal comprises using a fluorometer.

51. The method of claim 30, wherein the sensor comprises multiple quantum dots, chromoionophore and ionophore.

52. The method of claim 51, wherein the sensor is a film.

53. The method of claim 30, wherein the fluorescence source comprises one or more quantum dots.

54. The method of claim 30, wherein the fluorescence source comprises one or more fluorescent dyes.

55. The method of claim 30, wherein the sensor further comprises a targeting moiety.

56. The method of claim 55, wherein the targeting moiety is bound to any of the polymer matrix, the surface modifier, the internalizing moiety or a combination thereof.

57. The method of claim 55, wherein the targeting moiety is selected from small molecules, proteins, sugars or any combination thereof.

58. The sensor of claim 55, wherein multiple targeting moieties are bound to one or more components of the sensor.

59. The method of claim 30, wherein the internalizing moiety is bound to any of the polymer matrix, the surface modifier, the targeting moiety or a combination thereof.

60. The method of claim 30, wherein the internalizing moiety is selected from an amine, antepennepedia, mastoparan, melittin, bombolittin, delta hemolysin, pardaxin, *Pseudomonas* exotoxin A, clathrin, Diphtheria toxin, C9 complement protein, or a fragment of one of the preceding proteins, or a combination thereof.

61. The method of claim 30, wherein multiple internalizing moieties are bound to one or more components of the sensor.

62. The method of claim 30, wherein the internalizing moiety also functions as a targeting moiety.

* * * * *